放影研 RERF update
Radiation Effects Research Foundation News and Views
Hiroshima and Nagasaki, Japan
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Table of Contents

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RERF conducts research and studies—for peaceful purposes—on the medical effects of radiation on humans with a view toward contributing to the maintenance of the health and welfare of atomic-bomb survivors and to the enhancement of the health of all mankind.

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Technical Editor: *Yuko Ikawa, Public Relations & Publications Office*

Editorial Policy

Contributions to Update *receive editorial review only and do not receive scientific peer review. The opinions expressed herein are those of the authors only and do not reflect RERF policies or positions.*

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From the Editors

Haikei!

Well, here we are once again…trying to catch up on our reporting of RERF's various research activities over the past several years.

Faithful readers…gambatte kudasai (keep your chin up)…we're close to our objective! More importantly, we're trying to…and believe that we are succeeding…in making significant improvements in the quality of our reports, as well as making the reports more enjoyable to the reader.

Toward this end, we have included in this volume three very interesting and timely science articles: the first by Dale Preston and colleagues, concisely and elegantly summarizes the findings of their recent landmark study in which radiation-associated cancer (solid tumors) risks were updated, documented, and characterized for various organ systems of body; the second article by Waka Ohishi, reports on a technical breakthrough in using archived frozen sera diagnostic virology; and the third article by Hidetaka Eguchi, summarizes his recent findings on the molecular biology (genomic instability) of radiation-associated colorectal cancer in A-bomb survivors.

In addition, we have included a "Facts and Figures" piece on the Information Technology Department's "Data Processing and Management System for Adult Health Study" (by Hiroaki Katayama). Also, we would like to point out a special "Human Interest" article that we believe that you will enjoy, namely an interview with five very distinguished RERF retirees who recalled their early days working at ABCC in Hiroshima and Nagasaki.

So as always, please enjoy this volume, and of course feel free to drop us a line and let us know your thoughts on our reporting of RERF's many activities.

Sincerely,

Thomas M. Seed
Editor in Chief

Yuko Ikawa
Technical Editor

Letter to the Editor

I regularly read "RERF Update" and am very impressed with its continuingly improved quality. It is clear that all those involved in the creation of this publication are continuously striving to achieve an impeccable product. The recent addition of 'Science Articles' and 'Human Interest Notes' are especially noteworthy. I have read the 'Science Articles' of the last two volumes (16 and 17) and am thrilled by the standard of published papers.

In volume 17 the first science article titled "The frequency of XPA heterozygotes bearing a founder mutation among individuals who resided in Hiroshima and Nagasaki" by Hirai *et al*. has elegantly investigated the frequency of heterozygotes bearing a specific mutation in the *Xeroderma pigmentosum group A (XPA)* gene in a cohort from Hiroshima and Nagasaki. XP is a rare autosomal recessive disorder with high incidence of UV-related skin cancer associated with the impaired ability to repair UV-induced DNA damage due to a defective translesional DNA polymerase. The authors have developed a simple polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) in their laboratory to identify XPA heterozygotes bearing the founder mutation in a cohort of 1,020 individuals who resided in Hiroshima or Nagasaki. Primer set extending into intron 3 and exon 4 of the XPA gene (the DNA region that includes the founder mutation) was used for PCR amplification and the amplification product was a 61 bp fragment. If the founder mutation was present, the amplified 61 bp DNA was cleaved into two fragments (25 and 36 bp) with *Alw*N1 digestion. This is the first molecular genetic, population based study focusing XP heterozygotes. It demonstrated that the frequency of XPA heterozygotes bearing the founder mutation was 1 in 113 (9 out of 1,020; 0.88%) among Hiroshima and Nagasaki population. This estimate suggests that there may be approximately 1 million carriers of the XPA founder mutation in the 120 million population of Japan. The frequency of XPA heterozygotes observed in this study suggests that the risk of skin cancer in XPA heterozygotes can be elucidated.

The second science article in volume 17 is by Cullings *et al*. and titled "The introduction of dosimetry system DS02 and its impact on survivor doses and related risk estimates." This article states that the new dosimetry system, DS02, makes a more accurate estimate of the received dose because it takes more factors into account. It improves upon

the old DS86 by taking into account leakage in the casing of the bombs that would lower the amount of radiation given off, the shielding effect of low structures, which were ignored by DS86, and the shielding of various pieces of furniture *inside* buildings, enhancing the "globe" method of shielding calculation, as well as taking into account the shielding from the terrain (features such as hills). The authors noted that only the terrain shielding produced a substantial effect on the doses received, whereas all the other modifications made only slight changes. Furthermore, DS02 improves on determining the bodies self shielding effect for neutron radiation, as seen by $T_{body,n}$ being about 6% to 9% less in DS02 than in DS86. The DS02 system makes use of GIS with maps, aerial photography, and survivor shielding history drawings to better predict the correct dose received by a person exposed to radiation than the previous system.

Dr. Vijay K. Singh
AFRRI (Armed Forces Radiobiology Research Institute)
Bethesda, MD, USA

Report on the 33rd Scientific Council Meeting

The 33rd Scientific Council meeting, co-chaired by Dr. Clarice Weinberg (U.S. National Institute of Environmental Health Sciences) and Dr. Ohtsura Niwa (Kyoto University), was convened on March 27–29, 2006 in Hiroshima to review the scientific program of RERF. The Council meeting was opened by Vice Chairman Dr. Roy E. Shore's announcement and memoriam of the death of Dr. Robert Miller, who was an eminent scientist in the fields of pediatrics, genetics and cancer epidemiology and made a number of outstanding scientific contributions in the early days of ABCC. Dr. Toshiteru Okubo, the Chairman of RERF, then welcomed the Councilors and reported on administrative issues faced by the Foundation over the past year. Thanks was extended to three executive members, Dr. Burton G. Bennett, Dr. Eiichi Tahara, and Dr. Senjun Taira, whose terms of service were completed in the last year. Dr. Okubo discussed the budget cut by the Japanese government, which inevitably will necessitate reducing the number of personnel, possibly affecting future activities of the Foundation. Dr. Okubo also announced that, following the recommendation of last year's Scientific Council, a Blue Ribbon Panel will convene later this year to review and make recommendations regarding the status and future of RERF's scientific programs. This is a particularly timely action, given the multiple challenges and issues faced by the Foundation. Subsequently, overviews of RERF departmental research and several more detailed reports of new research findings were presented, along with a presentation by Director Takanobu Teramoto on RERF's newly upgraded public relations and communications program. Councilors met informally with individual departments on the second afternoon.

The Scientific Council expressed endorsement of the core mission of RERF and support for research in line with its mission. It was impressed by progress made by RERF since the last year and acknowledged the positive actions taken by RERF in response to the recommendations made by the Council the previous year. The Council made a number of general recommendations as well as ones applicable to the research programs in specific departments. The principal general recommendations and several departmental ones with general implications were:

- Interdepartmental communication and data sharing are improving but more attention to this will enhance the utility of information and scientific productivity, especially considering that complex diseases and systems biology are increasingly important. Develop methods to link RERF data sources, while respecting the constraints of the new privacy laws.
- They applauded the recent establishment of a monthly colloquium as a mechanism for openly discussing work-in-progress in a highly informal and multidisciplinary context.
- The process of evaluating proposed Research Protocols needs to be streamlined if this can be done without sacrificing quality.
- They encouraged the development of mechanisms to reward and improve research productivity of each of the scientists.
- Improvements need to be made in electronic access to scientific journals.
- Careful attention should be paid by the Foundation to past recommendations.
- Scientists should be encouraged to apply for outside grants to support research, insofar as they fit well within the mission of RERF. This will help to enhance the visibility of the organization, build synergistic collaborative relationships, and improve the science.
- Recruitment remains a critical issue in all departments in RERF, particularly given that senior scientists are coming to retirement ages. Those individuals have played crucial roles as leaders in the research and often as the interface to the outside radiation community. Efforts

should be made to find creative ways to forestall impending losses of key scientists due to governmental policies on mandatory retirement. Recruitment of younger investigators is especially critical for the future of the Foundation.
- They strongly endorsed the partnerships RERF has established in epidemiology and biostatistics with the University of Washington and Kurume University. This kind of relationship (and its future extensions) can benefit the radiation research community by promoting collaborations and developing scientists who are knowledgeable in radiation risk assessment.
- Try to increase communication and collaboration with other RERF departments in order to acquire relevant statistical, clinical, epidemiologic and/or basic-research expertise early in the design and conduct of the research.
- Efforts should be continued on the F_1 cohort studies with wide and sustained collaboration. These studies are particularly important for examining the prevalence of various non-cancer diseases.
- Efforts were applauded to increase the size of the Adult Health Study cohort for those who were under the age of five at the time of the bombing.
- The positive relationship between radiation and non-cancer diseases and conditions calls for studies to clarify the underlying mechanisms.
- The update of the papers on cancer incidence in the Life Span Study and in the *in utero* exposed cohort will be of wide scientific interest and should be submitted to high impact journals.

They suggested that the pending Blue Ribbon Panel:
- Address the long-term mission of RERF, as survivors expire (around 2040) and the F_1 cohort drops below 10% around 2055.
- Assess the assumption that at that point in time radiation effects will still be relevant to science and society.
- If radiation health effects are deemed important, consider themes for a future RERF, such as: 1) preparedness for nuclear accidents or terrorism and hazards of medical irradiation; 2) human systems biology (gene-environmental interactions in the origins of disease); and 3) risk assessments for environmental hazards to health, including the role of the human genome in modifying responses to environmental factors.
- Address logistic details for supporting a reaffirmed RERF, such as: binational versus multinational sponsorship, sources of funding, etc.
- Propose a long-term strategy to maintain the prestigious role of RERF and its leadership in the radiation risk research communities.

RERF Scientific Councilors

Dr. Ohtsura Niwa, Professor, Radiation Biology Center, Kyoto University, *Co-chairperson*
Dr. Clarice Ring Weinberg, Chief, Biostatistics Branch, Environmental Diseases and Medicine Program, National Institute of Environmental Health Sciences, *Co-chairperson*
Dr. Shinkan Tokudome, Professor, Health Promotion and Preventive Medicine, Nagoya City University Graduate School of Medical Sciences
Dr. Teruhiko Yoshida, Chief, Genetics Division, National Cancer Center Research Institute
Dr. Yoshiharu Yonekura, Director, Biomedical Imaging Research Center, University of Fukui
Dr. Katsushi Tokunaga, Professor, Department of Human Genetics, Graduate School of Medicine, University of Tokyo
Dr. Joel S. Bedford, Professor, Department of Radiological Health Sciences, Graduate Faculty of Cellular and Molecular Biology, Colorado State University
Dr. Theodore L. DeWeese, Professor and Chair, Department of Radiation Oncology and Molecular Radiation Sciences, The Johns Hopkins University School of Medicine
Dr. Marianne Berwick, Professor and Chief, Division of Epidemiology, Department of Internal Medicine, University of New Mexico
Dr. John J. Mulvihill, Professor of Pediatrics, University of Oklahoma Health Sciences Center

The 41st Board of Directors Meeting in Hiroshima

The 41st meeting of the Board of Directors was held at the RERF Auditorium during June 21–22, 2006. Valuable opinions were actively exchanged regarding RERF's future vision for the next 10–20 years, including the issues of personnel reduction requested by the Japanese government and budgetary actions. In particular, in relation to the "Senior Review Panel on Future Planning for RERF," to be established in autumn of the same year, RERF requested the Board members to provide advice regarding the Foundation's future vision. Many of the visiting directors present at the Board meeting

stated that RERF should continue its program as a research institution, rather than as a radiation information center.

RERF's future vision has been repeatedly discussed as one of the organization's top-priority issues. During this year's Board meeting, there was lively discussion regarding this issue, as well as about the daunting challenges facing RERF. The main topics discussed during the two-day meeting are briefly described below.

In his status report on RERF at the beginning of the meeting, Dr. Toshiteru Okubo, RERF Chairman, stated that negotiations about the U.S.-Japan five-year agreement on the management of RERF were held in Tokyo in November 2005 and that establishment of a new Senior Review Panel during 2006 for making recommendations regarding the future of RERF was included in the agreement. He also reported that at the first meeting of the Forum for Institutes Engaged in A-bomb Survivors' Medical Care held in May 2006 in Hiroshima at the request of Dr. Shizuteru Usui, President of the Hiroshima Prefectural Medical Association, proposals were made to further strengthen the partnership among local institutes engaged in medical care for A-bomb survivors and that the results of deliberations by the Forum would be conveyed to the Senior Review Panel as opinions representing the local community.

Thus, arrangements are being made to provide an opportunity for the U.S. and Japanese governments to discuss RERF's future vision, while efforts are under way to collect and summarize relevant opinions of the local community. Behind these activities is the harsh reality facing RERF. What follows is a report from the RERF side regarding its difficult situation and proposed countermeasures.

During the next four years, employment capacity will be decreased by six persons per year. The salaries of RERF employees will be decreased according to the rate of decrease applied to government employees. However, the most important policy for continuation of RERF's research activities is the retaining of research staff. With this in mind, every effort has been made to minimize the effects of the current situation on research activities. Measures taken include (1) maintenance of the existing fixed number of research scientists, (2) preparation of medium- to long-term personnel plans for recruitment of leading research scientists, and (3) introduction of a new system for employment of research scientists with fixed terms of appointment.

With regard to the above report about RERF and its difficult set of circumstances, some of the visiting directors voiced their opinions that, since RERF is a research institution jointly funded by the U.S. and Japan, the Board should discuss how U.S. government ideas regarding RERF can be better reflected in the management of the Foundation, and that, since U.S. funds account for 40% of all its operational funds, RERF should request the Ministry of Health, Labour and Welfare (MHLW) to exempt RERF from across-the-board application of this policy of personnel reduction. It was decided that the results of deliberations of the Board of Directors would be conveyed to the Japanese government.

It was also reported at the meeting that, although the number of participants in the Adult Health Study (AHS), a program of major interest in future plans for research, will decline to almost zero around 2040, AHS has provided useful information regarding human health, unavailable from studies of mortality or cancer incidence only, and that long-term continuation of the program would be meaningful.

As for public relations activities to increase awareness among the public inside and outside the country regarding RERF's research activities, it was reported that, in accordance with the recommendations of last year's Scientific Council meeting, the Public Relations and Publications Office was established in the Hiroshima Laboratory and a person in charge of public relations was placed in the Nagasaki Laboratory, to ensure that PR activities are strengthened and PR strategies established.

With regard to the FY2006 research activities report, there was a question about whether or not the F_1 Clinical Study (FOCS) should be continued as a longitudinal study. In response to this, some of the visiting directors, while admitting that FOCS was an invaluable study, pointed out the necessity of conducting close review before starting the longitudinal study.

Finally, a discussion ensued concerning election/appointment of officers, with Dr. Takashi Yanagawa (professor with special appointment, Biostatistics Center, Kurume University) and Dr. David G. Hoel (distinguished university professor, Department of Biometry and Epidemiology, Medical University of South Carolina) elected as successors for two Scientific Councillors (Dr. Shinkan Tokudome and Dr. Clarice Ring Weinberg), whose terms were set to expire June 30, 2006. The terms of office of the new Scientific Councillors will be five years, to June 30, 2011.

It was decided that the next Board of Directors meeting would be held at the Nagasaki Laboratory for the three days from June 20 to 22, 2007.

As a whole, at this year's Board meeting, there were many items that could not be discussed without future plans in place. In this sense, establishment of the Senior Review Panel, which was strongly recommended by the Scientific Council last year and for which the Board of Directors expressed support last year, is attracting much attention, because the panel will have a large impact on the future of RERF.

List of Participants

Directors:
Dr. Toshiteru Okubo, Chairman
Dr. Roy E. Shore, Vice Chairman and Chief of Research
Mr. Takanobu Teramoto, Permanent Director
Mr. Masaaki Kuniyasu, Former Ambassador Extraordinary and Plenipotentiary to the Republic of Portugal
Dr. Yasuhito Sasaki, Vice President, International University of Health and Welfare
Dr. Senjun Taira, Permanent Director, Japanese Association of Quarantine Inspection Hygiene
Dr. Paul L. Ziemer, Professor Emeritus, Purdue University
Dr. James D. Cox, Professor and Head, Division of Radiation Oncology, University of Texas M.D. Anderson Cancer Center
Dr. John E. Burris, President, Beloit College

Supervisors:
Dr. Tomio Hirohata, Professor Emeritus, Faculty of Medicine, Kyushu University
Mr. David Williams, Senior Financial Advisor, National Academy of Sciences

Scientific Councilor:
Dr. Ohtsura Niwa, Professor, Radiation Biology Center, Kyoto University

Representatives of Supporting Agencies:
Mr. Katsunori Hoshi, Chief, Relief Budget Unit, General Affairs Division, Health Service Bureau, Ministry of Health, Labour and Welfare
Dr. Joseph F. Weiss, Japan Program Manager, Office of Health Studies, U.S. Department of Energy
Mr. Yuriy R. Fedkiw, Science and Technology Officer, Embassy of the United States of America (acting Minister-Counselor for Joyce Rabens)
Dr. Kevin D. Crowley, Director, Board on Nuclear and Radiation Studies, Division on Earth and Life Studies, National Research Council, National Academy of Sciences
Dr. Evan B. Douple, Scholar, Board on Nuclear and Radiation Studies, Division on Earth and Life Studies, National Research Council, National Academy of Sciences

RERF:
Mr. Eiji Akimoto, Chief of Secretariat
Dr. Thomas M. Seed, Associate Chief of Research
Dr. Kazunori Kodama, Chief Scientist/Chief, Department of Epidemiology
Dr. Nori Nakamura, Chief Scientist/Chief, Department of Genetics
Mr. Douglas C. Solvie, Associate Chief of Secretariat

Staff News

As in any organization change is inevitable; RERF is no exception. The scientific staff continues to evolve, with new faces appearing, old faces disappearing, and some faces simply changing features. During the period from April 2006 to March 2007, there have been four new additions to the scientific staff, along with four departures. In addition, three long term employees retired, but were subsequently rehired under new contracts of employment. First, in terms of the new "faces," Dr. Phillip Ross arrived in August of last year, leaving his former consulting and teaching posts in the U.S., and assumed his new role here as Chief of Statistics. We feel very fortunate to have Dr. Ross onboard, as he has considerable managerial experience and an academic background in a wide-range of statistical analyses. In addition, Dr. Kengo Yoshida and Dr. Ritsu Sakata, both bright, highly motivated, and energetic researchers, joined RERF in April of 2006 and are now gainfully employed in the Department of Radiobiology/Molecular Epidemiology and the Departments of Statistics and Epidemiology, respectively. We fully expect to see great things coming from the work of these scientists. The Clinical Studies Department has also benefited from the arrival in April (of 2006) of a more seasoned physician/clinical researcher, namely Dr. Ikuno Takahashi. Dr. Takahashi's medical specialty is in internal medicine with a clinical research focus in the area of cardiovascular disease.

So much for the "new faces"; what about the "changing faces." There are three: Dr. Nori Nakumra, Mimako Nakano, and Kazuo Ohtaki. All of these staff members spent sizable fractions of their careers here at RERF working in the area of cytogenetics. Following their retirements, all were reemployed under new guidelines established here at RERF. Dr. Nakano and Dr. Ohtaki have been rehired as Adjunct Research Specialists within the Cytogenetics Laboratory, whereas Dr. Nakumara has assumed a range of administrative duties under the title of Chief Scientist. Regardless of the mechanism by which these very talented and experienced researchers were reemployed, we are delighted that we were able to retain these individuals and their most valued services.

During this period, however, RERF lost some

outstanding researchers who over the years made significant contributions to the RERF's science. Dr. Kiyoto Ashizawa, very talented clinical researcher in the Department of Clincial Studies in Nagasaki, received and accepted a very nice offer of a position as Chief of the Endocrinology Department at Saiseikai Hospital in Nagasaki. In addition to Dr. Ashizawa, the very prominent cancer scientist, Dr. Eiichi Tahara, who was serving as "Senior Consulting Scientist" resigned his post in June of 2006.

It should be noted that Dr. Tahara ably served RERF in various senior administrative capacities, including Permanent Director and Chief of Research. His foresight on a number of leading-edge biomedical research areas will be missed.

Honors and Awards Received by RERF Scientists

There is an American saying, "When it rains, it pours," and RERF investigators received a virtual cloudburst of honors. In the fall of 2006 no fewer than five RERF researchers won important awards for their research contributions from various scientific societies in Japan and abroad. Below, each researcher will briefly describe the award he or she received.

In addition to the above scientific awards, Dr. Kazunori Kodama, RERF Chief Scientist, was appointed Chairman of the Japan Epidemiological Association (JEA) for the term of three years at the JEA's 17th annual meeting held in Hiroshima on January 26–27, 2007, for which he served as chairman. Dr. Kodama's appointment is an honor also for RERF, which has focused on epidemiological research over many years, and we are pleased to report this news in the *RERF Update*.

Excellent-presentation Award at the 49th Meeting of the Japan Radiation Research Society

Kanya Hamasaki, Department of Radiobiology/Molecular Epidemiology

I received an excellent-presentation award at the 49th meeting of the Japan Radiation Research Society, held at Hokkaido University for three days from September 6–8, 2006. The excellent-presentation award was first established at this meeting and was presented to the 12 posters of distinguished quality selected from among a total of 192 posters with presentation themes representing the various fields related to radiation research (radiation effects, radiation response, lesion/repair, radio-therapeutic biology, radiation epidemiology, environmental effects, among others). At the meeting, I made a presentation about the "Study on genetic instability in peripheral blood lymphocytes among the A-bomb survivors," in which we cloned A-bomb survivor peripheral blood T cells *in vitro* and analyzed, with the M-FISH method, each of the clones obtained for determination of frequency of additional chromosomal mutations occurring in the cultivation process with the aim of studying genetic instability.

Currently working as an associate senior technician at the Cell Biology Laboratory, Department of Radiobiology/Molecular Epidemiology, I have been working as a returning student at the Division of Integrated Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, since April 2003 (a joint program between RERF and Hiroshima University). Since starting my study at Hiroshima University, I have been conducting research based on the theme "Study of genetic instability induced by radiation exposure," with tutelage and support from Dr. Yoshiaki Kodama (Genetics Department), Dr. Yoichiro Kusunoki (Radiobiology/Molecular Epidemiology Department), and related technicians. I would therefore like to take this opportunity to express my gratitude to these special individuals.

First Morii Award from the Japan Osteoporosis Society

Saeko Fujiwara, Chief Department of Clinical Studies, Hiroshima

I was honored to receive the first Morii Award from the Japan Osteoporosis Society (JOS) at its FY2006 meeting, held in Tokyo over three days, from October 12 through 14. This award is based on a donation made by the family of Dr. Kosei Morii, who was the chairman of JOS and passed away last year, and is presented to a principal author of the most distinguished clinical research paper among the JOS members. The paper selected for the award was "Heel bone ultrasound predicts non-spine fracture in Japanese men and women," *Osteoporosis Int* 16:2107–12, 2005.

Although ultrasound bone assay had been widely used for examination of osteoporosis in Japan, no reports had been published regarding whether or not the technique could be used to predict fracture risk. Evidence for the Japanese population was acquired for the first time in our study, and that was the major reason for this work being selected for the award. This study was conducted jointly by RERF,

Kawasaki Medical College, and Hamamatsu Medical College, and I served as coordinator of the study. The study, however, could not have been completed without the considerable efforts made by the late Dr. Shoichiro Fujita, who conducted the statistical analysis, and those of Ms. Naomi Masunari, research assistant of the Department of Clinical Studies.

Hypertension Research-Novartis Award for Best Research Paper

Masazumi Akahoshi, Chief
Department of Clinical Studies, Nagasaki

The 21st meeting of the International Society of Hypertension was held in Fukuoka on October 15–19, 2006. At the general meeting of the Japanese Society of Hypertension (JSH), held during the same period, on October 18, I received the sixth Hypertension Research-Novartis award for the best research paper. This award is presented to the best research paper of the year selected from among the papers published in the JSH newsletter *Hypertension Research*. The paper for which I was presented with the award was titled "Basic characteristics of chronic hypotension cases: A longitudinal follow-up study from 1958 through 1999." As the title of the paper suggests, our study was based on data accumulated in the Adult Health Study.

This study was conducted to ascertain if analyzing the characteristics of those whose blood pressure did not increase with age would further clarify the factors involved in advancing blood pressure with age. As a result, we found 92 individuals who had lived long with chronic hypotension and whose blood pressure had not advanced with age. Their characteristics were slight build, slow pulse, low body temperature, and anemia. It was considered that these characteristics were induced by decreased tension of the sympathetic nervous system, and from this, it was further assumed that the blood pressure increase with age seen among modern individuals was not biological inevitable, but something originating from increased tension of the sympathetic nervous system.

JSH Award from the International Society of Hypertension

Yoshimi Tatsukawa, Research Scientist
Department of Clinical Studies, Hiroshima

The 21st meeting of the International Society of Hypertension (ISH 2006) was held in Fukuoka during October 15–19, and I received the JSH award, which is presented to a young Asian researcher, for our study "Relationship between white blood cell count and incidence of hypertension among a Japanese population." This society, which meets every two years, was held in Japan for the first time in 18 years, and this time it was convened jointly with the fifth meeting of the Asian and Pacific Society of Hypertension (APSH) and the 29th meeting of the Japanese Society of Hypertension (JSH). At this last meeting of ISH, quite a few awards were established for young researchers, including the award presented to me.

It has been suggested in recent years that chronic inflammation is involved in arteriosclerosis as a causal factor. In our presentation, we used white blood cell (WBC) counts (an inflammatory marker) from the Adult Health Study (AHS) population in 1965–67, and followed up the association between WBC counts and development of hypertension for a long period of about 40 years. Almost no other study exists in which a follow-up of a large-scale population like that of AHS has been conducted, and I feel that the society considered this point when deciding to present the award to us.

Poster Prize at the International Association of Cancer Registries

Nobuo Nishi, Chief, Tumor and Tissue Registry Office
Department of Epidemiology, Hiroshima

I attended the 28th Annual Meeting of the International Association of Cancer Registries, held in Goiania, Brazil, from November 7 to 10, 2006, and my presentation was awarded a poster prize. Unlike most other international association meetings, this association's meeting is held every year, usually in a developing country. Also attending from RERF were Dr. Midori Soda (executive member for the association's Asian region), Assistant Chief of the Department of Epidemiology in Nagasaki, and Dr. Hiroaki Katayama, Chief of the Department of Information Technology.

My poster titled "Socioeconomic differences in cancer mortality, incidence and survival in Japan" was the first one introduced during the presentation of poster awards. It was indicated that my poster was given high marks because it was well organized, effectively using tables albeit with no graphs.

Dr. Hans Storm from Denmark, one of the poster judges, said that he had read abstracts prior to the meeting and viewed mainly posters with the most intriguing contents, in order to choose several posters to be awarded from among nearly 100.

Results of the Health Effects Study of the Children of A-bomb Survivors Announced

Akihiko Suyama, Chief, Department of Epidemiology, Nagasaki

RERF has conducted for seven years from 2000–2007 a health effects study of the children of A-bomb survivors to investigate whether there is any relationship between the prevalence rates of adult-onset multi-factorial diseases (hypertension, diabetes mellitus, hypercholesterolemia, myocardial infarction, angina pectoris, and stroke) and parental radiation exposure.

It is considered that multi-factorial diseases develop due to both genetic factors and lifestyle factors. Since the prevalence rates of multi-factorial diseases increase in adulthood, it is meaningful to try to evaluate the association of these diseases with underlying genetic events of radiation-exposed parents. To our knowledge, this health effects study is the first to investigate such association in humans.

In planning, and later conducting this study, committees of outside experts were established to investigate the appropriateness of study objectives and plans as well as the ethical issues involving study methods. These committees included: the Scientific Committee (chairman: Dr. Tadao Shimao, Consultant to the Japan Anti-Tuberculosis Association), the Ethics Committee (chairman: Dr. Hiraku Takebe, Visiting Professor of Kinki University Interdisciplinary Graduate School of Science and Technology), and the Analysis Subcommittee (chairman: Dr. Norihiko Hayakawa, Professor Emeritus of Hiroshima University). Thus far, there have been five meetings of the Scientific Committee, four meetings of the Ethics Committee, six joint meetings of the Scientific and Ethics Committees, and four meetings of the Analysis Subcommittee.

At the fourth meeting of the Analysis Subcommittee held on February 27, 2007, it was reported that the initial goal of 10,000 clinical exam participants had been achieved (more precisely: 11,951, a participation rate of 48.4% out of 24,673 for whom the mail survey was conducted), thereby generating data with sufficient statistical power. The report concluded that analysis of association between radiation exposure and a combination of six multifactorial diseases (including diabetes and hypertension, conducted by taking into account such lifestyle factors as drinking and smoking) failed to detect increased risk. It was also reported that a negative association was observed between paternal dose and prevalence rate of these multi-factorial diseases, which was significantly low, among male children. However, it was considered that careful interpretation of this finding would be necessary.

While the Scientific and Ethics Committees accepted the analysis results of the Analysis Subcommittee, they recommended that the clinical health study of the fixed population be continued in a prospective manner, taking into account that the average age of the study population was still young, at 48.6 years. This recommendation was made despite the fact that no association was identified between parental exposure and health effects among the offspring. As the RERF's health effects study of the A-bomb survivors' children had attracted much public attention since its start, the above conclusions were explained to the press on February 28, using a videoconferencing system connecting Hiroshima and Nagasaki and reported nationwide.

Thus, the study conducted in a cross-sectional format for seven years since 2000, marked the end of one stage of what is anticipated to be a continuing evaluation. We would like to express our heartfelt appreciation to the committee members for their dedication to the study, as well as to the children of A-bomb survivors for their kind cooperation.

Solid Cancer Incidence among Atomic Bomb Survivors, 1958–1998

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Although a great deal is already known about the carcinogenic risks from acute exposure to external radiation, the long-term follow-up of the Life Span Study (LSS) of atomic-bomb survivors continues to provide new insights into and raises additional questions about the nature of radiation effects on cancer risk. We have just published the second comprehensive report on solid cancer and nervous system tumor incidence in the LSS.[1] (Solid cancer includes all malignancies other than leukemia and other malignancies of the lympho-hematopoietic system.) The main objectives of our study were to quantify cancer risks attributable to radiation; to explore the shape of the dose-response; to assess how risk is modified by age, time, gender and other factors; to help clarify site-specific differences in risk patterns; and to identify issues and cancer sites needing further research.

The LSS cancer incidence studies are an important complement to the analyses of cancer mortality in the cohort because they include much more data on less-fatal cancers, such as cancers of the breast, thyroid and skin, which are radiation sensitive, but are under-represented on death certificates. Tumor registry diagnoses are based on a review of data from many sources and are substantially more accurate and detailed than cause-of-death data gleaned from death certificates. Furthermore, the date of cancer incidence is a better indicator of disease onset and biologically more relevant than date of death from cancer which is influenced by stage at diagnosis and treatment.

With follow-up from 1958 through 1998, the new report adds 11 years of follow-up, and many more cancer cases. In contrast to the first LSS cancer incidence report in 1994,[2] the so-called "not in city" (NIC) group was included in the new analyses because the addition of about 25,000 cohort members considerably improves the precision of the descriptions of baseline cancer risk patterns. With this addition and the extended follow-up time, the study includes 24% more person-years and 56% more cancer cases than the 1994 report.

The LSS cohort includes 120,321 people who had been born at the time of the bombs and were alive and living in Hiroshima or Nagasaki in late 1950, but who were not otherwise selected on the basis of health status. About 95,000 cohort members were within 10 km of the epicenter and about 25,000 were

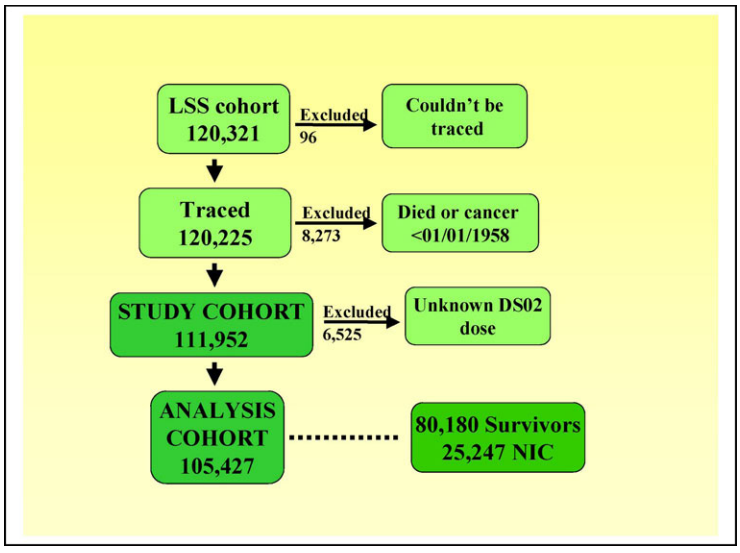

Figure 1. The Life Span Study Cohort: description of how the analytic cohort was defined.

Table 1. Life Span Study cancer incidence analysis cohort*: percentage surviving on December 31, 1998 by age-at-exposure and gender

Age at exposure	Male		Female		Total	
	People	% alive	People	% alive	People	% alive
<10	11,196	89%	11,478	94%	22,674	91%
10-19	10,374	74%	12,689	87%	23,063	81%
20-29	3,299	47%	10,964	72%	14,263	66%
30-39	5,224	17%	10,595	36%	15,819	30%
40-49	6,912	1%	9,170	5%	16,082	4%
≥50	5,897	0%	7,629	0%	13,526	0%
All ages	42,902	47%	62,525	54%	105,427	51%

*Limited to people who were alive and reported to be cancer free as of January 1, 1958

NIC at the time of the bombings. The new incidence analyses were based on 105,427 LSS cohort members who have estimated DS02 organ doses and who were alive and not known to have had cancer on January 1, 1958 (Figure 1). As indicated in Table 1, 51% of the study cohort members were alive at the end of follow-up on December 31, 1998. Slightly more than 40% of the cohort members were younger than 20 years of age at the time of the bombings, and about 85% of these cohort members were alive at the end of follow-up.

The whole body doses received by individual LSS cohort members ranged from no exposure to more than 4 Gy, with a mean dose of about 0.25 Gy among cohort members with doses of 5 mGy or more. The analyses in this report were based on DS02 organ dose estimates. The DS02 dosimetry system, which was adopted by RERF in 2003, provides individual organ dose estimates for 95% of the cohort members.[3] DS02 incorporates refinements in the estimates of the bomb yields and source terms as well as new information on shielding effects on exposure.[4] These improvements resulted in slightly increased gamma doses and decreased neutron doses, but these changes have little impact on risk estimates.[5] Risk estimates for all solid cancers as a group were based on weighted colon dose estimates while site-specific analyses were based on doses to the relevant target tissue.

Cases were identified through the LSS Tumor Registry that includes all cancers and benign tumors of the nervous system diagnosed and reported to the Hiroshima and Nagasaki Tumor Registries since 1958 among LSS cohort members. The Hiroshima and Nagasaki Tumor Registries actively ascertain cancers in Hiroshima City and Nagasaki Prefecture from all large hospitals in their catchment areas and incorporate information from tissue registries that keep records of histological findings and the location of slides and other materials. The LSS Tumor Registry also includes information about cancer listed as the cause of death on death certificates for LSS members throughout Japan. This information is obtained through RERF's routine mortality follow-up. Commonly used indices of cancer registry quality indicate that the Hiroshima and Nagasaki registry data are of high quality with regard to the completeness and accuracy of diagnoses, and previous analyses demonstrated that there is no radiation dose bias in case ascertainment.[6]

Analyses were restricted to first primary tumors to prevent confounding from exposure to high-dose radiation from treatment for a first cancer and possible detection bias in cancer survivors who generally remain under close medical surveillance. The data were summarized in a highly stratified person-year (rate) table. The stratifying factors included city, gender, dose, age at exposure, attained age, and distance from the hypocenter. We used Poisson regression to estimate the baseline rates and the excess relative risks (ERR's) and excess absolute rates (EAR's) of all solid cancers combined and of individual cancer sites. The excess relative risk (ERR) describes the proportional change in the cancer incidence rate relative to the baseline (0 dose) rate as a function of dose and other factors (such as gender, attained age, or age at exposure). The excess absolute rate (EAR) describes the difference between the rate for an exposed group and the baseline rate for that group. ERR's are summarized as the ERR per Gy while EAR's have units of excess cases per 10,000 person-years per Gy. Since the ERR and EAR depend on dose and can vary with age at exposure, gender, attained age, and other factors, summary results are presented in terms of standardized values, typically the gender-averaged ERR (EAR) per Gy at age 70 for a person exposed at age 30.

In order to adjust for migration of LSS members out of the Tumor Registries' catchment area, we had to limit analyses to those cancers diagnosed within the catchment areas and we used gender, age, and time dependent residence probabilities to reduce person years. Residence probabilities were estimated using data on Adult Health Study exam contacting results.

Between January 1958 and December 1998, 17,448 eligible first primary solid cancers were identified among the 105,427 LSS cohort members included in the current analyses (Table 2). The mean age at diagnosis was 67.4 years and 54% of the cancers occurred among females, although 59% of

Table 2. Distribution of selected solid cancers identified between January 1958 and December 1998 among LSS cohort members

Site	Cases	% female	Mean age at diagnosis
TOTAL	17,448	54	67.4
Stomach	4,730	46	67.7
Lung	1,759	41	71.2
Colon	1,516	54	69.3
Liver	1,494	40	67.0
Breast	1,082	100	59.8
Cervix	859	100	60.0
Rectum	838	50	68.0
Bladder	469	33	70.6
Thyroid	471	81	60.4
Non-melanoma skin	330	63	72.4
Nervous system	281	67	62.6
Other	3,619	52	68.7

the cohort is female. As expected, stomach cancer, which has a very high incidence in Japan, was the most common cancer site. Lung cancer was the next most frequent cancer, but the number of cases was substantially smaller. Males predominated in both of these cancers. There also were over 1,000 cases of cancers of the colon, liver and breast.

Table 3 provides information on the dose distribution and cancer risks in the LSS. It can be seen that the dose distribution is highly skewed: dose estimates were less than 200 mGy for about 75% of the almost 45,000 cohort members with dose estimates above 5 mGy, whereas survivors with doses over 1 Gy account for less than 5% of survivors in this group.

The data in Table 3 also demonstrate a strong dose response and indicate that there are a considerable number of radiation-associated cancers (~160) in the 5 to 200 mGy dose range. At doses of 1 Gy or more almost half of the cancer cases identified among survivors were associated with their radiation exposure. For all solid cancers combined, we observed a linear dose response using weighted colon dose as the representative dose. There was a statistically significant dose response trend in the 0–0.15 Gy range which was similar to that estimated for the entire dose range (Figure 2).

Both the ERR per gray (ERR/Gy) and EAR per 10,000 person years per Gy (EAR/10^4 PY Gy) were about 50% higher for women than men. When gender-specific cancers were excluded from the analyses, the ERR/Gy remained significantly larger for females than males, but there was no gender difference using an EAR model. Figure 3 uses gender-averaged risks to illustrate how the excess risk varies with age-at-exposure and attained age. The ERR/Gy decreased with increasing age at exposure and attained age. The EAR/10^4 PY Gy also decreased with increasing age at exposure, but it increased with increasing attained age. Indeed, with a 25% increase in follow-up, we estimated a 50% increase in the number of radiation-associated cancer cases indicating that the radiation-effect on solid cancer incidence persists throughout life.

Statistically significant dose-responses were seen for most cancer sites, including oral cavity, esophagus, stomach, colon, liver, lung, non-melanoma skin, breast, ovary, bladder, nervous system, and thyroid (Figure 4). ERR's for cancers of the pancreas, prostate, and renal cell were non-significantly elevated but were consistent with the risk for all solid cancers as a group. Our data also suggest that the radiation-related risks for cancers of the rectum, gallbladder, and uterus may be lower than those for all solid cancers combined. There was evidence, however, that radiation exposure during childhood or adolescence may elevate the risk of developing cancer of the body of the uterus.

Assessing site-specific cancer risks is important because biologically it is almost certain that variation in site-specific risks exists. But, even in a study of over 100,000 people, the number of cases for most

Table 3. LSS solid cancers 1958–1998. Subject, person-year, and cases with fitted-excess and attributable-fraction estimates by dose category.

Dose category †	Subjects	Mean distance (m)	Person years	Cases	Fitted excess	Attributable fraction (%)
Not in city	25,427	–	680,744	3,994	0	0
< 0.005	35,545	3969	918,200	5,603	3	0.0
0.005 – 0.1	27,789	2114	729,603	4,406	81	1.8
0.1 – 0.2	5,527	1608	145,925	968	75	7.6
0.2 – 0.5	5,935	1430	153,886	1,144	179	15.7
0.5 – 1	3,173	1260	81,251	688	206	29.5
1 – 2	1,647	1118	41,412	460	196	44.2
2 +	564	934	13,711	185	111	61.0
Total	105,427		2,764,732	17,448	853	10.7‡

Note: Estimates of fitted excess cases are based on an ERR model with a linear dose response effect modification by gender, age at exposure and attained age. All not-in-city subjects were used in the modeling, but the baseline risk model allows for city-specific differences in the level of the baseline risks for the not-in-city group.

† Weighted adjusted colon dose in Gy.

‡ Attributable fraction among people who were in the cities with doses greater than 0.005 Gy

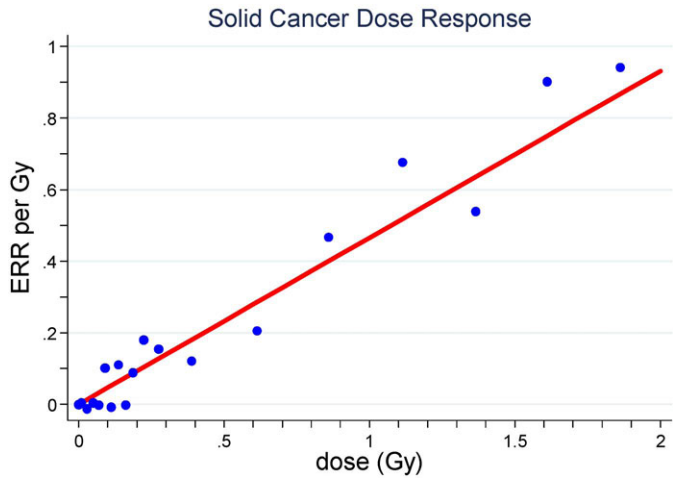

Figure 2. All solid cancer fitted linear dose response and dose category specific ERR estimates.

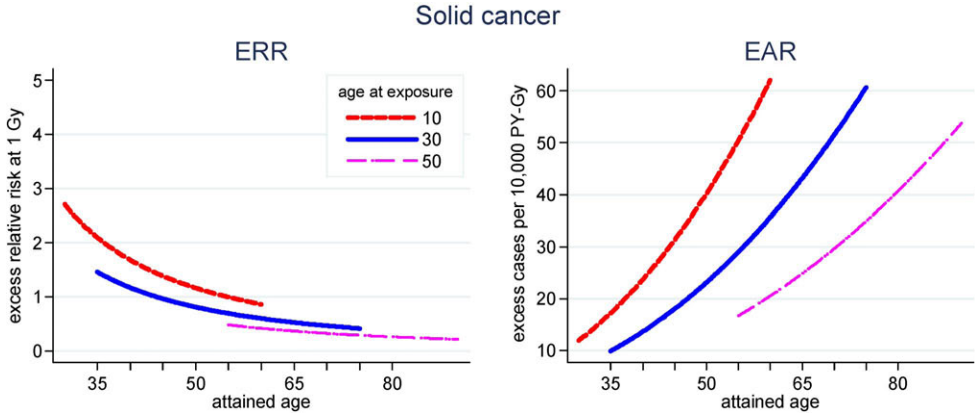

Figure 3. Age-dependence for the gender-averaged solid cancer ERR (left panel) and EAR (right panel) for exposure ages of 10, 30, and 50 years.

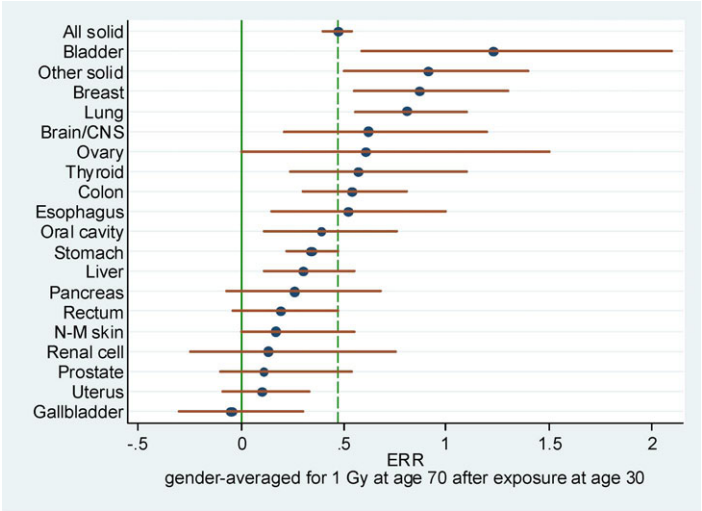

Figure 4. Site specific ERR estimates with 90% confidence intervals. The ERR's are gender-average and correspond to the fitted risk at age 70 for a person exposed to 1 Gy at age 30. The solid vertical line indicates no excess risk and the dashed vertical line represents the standardized ERR for all solid cancers as a group.

individual cancer sites is relatively small making it difficult to identify statistically significant differences in risk estimates or patterns. Although current analyses suggest some differences, much of the observed variability is consistent with random variation.

Figure 5 illustrates gender differences in the ERR estimates for selected sites. The large gender differences seen for bladder and lung cancer are particularly striking. To some extent the higher ERR for women than men for these smoking-related cancers reflects the relatively high prevalence of smoking among men in the LSS cohort and low prevalence among women. The gender difference for colon cancer is also remarkable and in the opposite direction (men had greater risks than women) for bladder and lung cancer. The reasons for this difference are unclear.

We also assessed radiation effects in five broadly classified histological groups, including adenocarcinoma, squamous cell carcinoma, other epithelial cancers, sarcomas, and other non-epithelial cancers. Over 96% of the solid tumors were epithelial tumors with adenocarcinoma accounting for about 60% of the epithelial tumors. ERR's per Gy of similar magnitude, from 0.3 to 0.48, were seen for the different histologic groups.

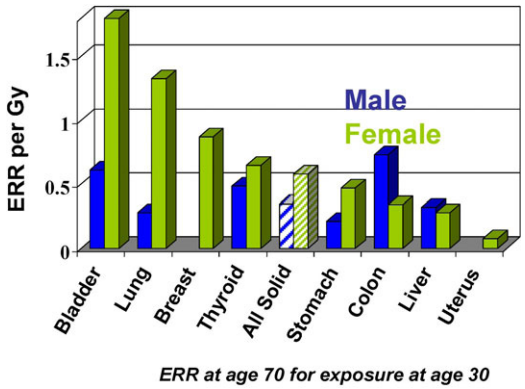

Figure 5. Gender-specific excess relative risk estimates for all solid cancers and selected sites.

In summary, more than half a century after the atomic bombings in Hiroshima and Nagasaki, a statistically significant increased risk of cancer incidence is still apparent among radiation-exposed members of the LSS. Among the survivors with doses of 5 mGy or more an estimated 10% of the solid cancers can be attributed to radiation exposure. A larger number of the radiation-associated excess solid cancers are expected to occur in the cohort over the next 15 to 20 years. The shape of the dose response for solid cancers as a group continues to be well-described by a linear model with no evidence of significant non-linearity. EAR's for all solid cancers combined increased throughout life for all ages, while ERR's decreased with increasing age at exposure and attained age. Excess risks for all solid cancers were higher for women than men, however when sex-specific cancers were excluded the difference in the EAR was reduced substantially. Lifetime solid cancer risk estimates were about 20 times higher than those observed in earlier studies of leukemia. While patterns of organ (or site)-specific risks generally were similar to those seen in the first cancer incidence study, they have become clearer for several cancers. High ERR's were found for cancers of the bladder, breast, and lung, whereas high EAR's were seen for cancers of the stomach, breast, colon, and lung. Accumulating data from further follow-up will continue to provide important new information on the levels and temporal patterns of the excess risk, especially for survivors younger than 20 years of age at the time of the bombings. Additional follow-up will also enable us to explore radiation-associated risks for rare cancer sites that didn't have adequate number of cases to study previously, and help clarify the variability in risk patterns for specific cancer sites. Finally, additional site-specific incidence studies incorporating pathology reviews and biomarker studies will add to our knowledge on the radiation-sensitivity of specific cell types.

References

1. Preston DL, Ron E, Tokuoka S, Funamoto S, Nishi N, Soda M, Mabuchi K, Kodama K. Solid cancer incidence in atomic bomb survivors: 1958–1998. *Radiat Res* 168:1-64, 2007.

2. Thompson DE, Mabuchi K, Ron E, Soda M, Tokunaga M, Ochikubo S, Sugimoto S, Ikeda T, Terasaki M, *et al.* Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958–1987. *Radiat Res* 137:S17-67, 1994.

3. Cullings HM, Fujita S, Funamoto S, Grant EJ, Kerr GD, Preston DL. Dose estimation for atomic bomb survivor studies: its evolution and present status. *Radiat Res* 166:219-54, 2006.

4. Young RW, Kerr GD, eds. *Reassessment of the Atomic Bomb Radiation Dosimetry for Hiroshima and Nagasaki—Dosimetry System 2002*. Radiation Effects Research Foundation, Hiroshima, Japan, 2005.

5. Preston DL, Pierce DA, Shimizu Y, Cullings HM, Fujita S, Funamoto S, Kodama K. Effect of recent changes in atomic bomb survivor dosimetry on cancer mortality risk estimates. *Radiat Res* 162:377-89, 2004.

6. Mabuchi K, Soda M, Ron E, Tokunaga M, Ochikubo S, Sugimoto S, Ikeda T, Terasaki M, Preston DL, Thompson DE. Cancer incidence in atomic bomb survivors. Part I: Use of the tumor registries in Hiroshima and Nagasaki for incidence studies. *Radiat Res* 137:S1-16, 1994.

Use of Stored Sera for Detection of Hepatitis B and C Viruses

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Introduction

Molecular biological research on hepatitis B virus (HBV) and hepatitis C virus (HCV) has advanced remarkably over the last decade, resulting in the establishment of methodologies for diagnosis, prevention, and treatment of liver diseases.[1–4] In addition, the use of dried blood samples (spot samples), as well as frozen sera, has enabled essential data to be collected for epidemiological and clinical studies, as well as for the screening of subjects with HBV or HCV infection.[5–8]

The Atomic Bomb Casualty Commission, the predecessor of the Radiation Effects Research Foundation (RERF), established the Adult Health Study (AHS) longitudinal cohort in 1958, comprised of approximately 20,000 atomic-bomb survivors and non-exposed controls that have been examined biennially in outpatient clinics in Hiroshima and Nagasaki. In 1969, Dr. Joseph L. Belsky, Department Chief of Medicine at the time, ordered additionally the assay of Australia Antigen, called HBV surface antigen (HBsAg) currently, for studies of liver function abnormality among AHS participants, however sera obtained from them had already been discarded. From 1969, sera obtained from the AHS participants have been stored by either freezing or freeze-drying methods with this as a turning point.[9] From 1990, their samples have been prepared and stored by both rapid freezing and by freeze-drying methods. In total, multiple serum samples from 16,000 subjects are currently stored at RERF, and represent a valuable resource for studies of the development of large variety of diseases.

Biochemical assays using frozen and freeze-dried sera showed that protein, lipid, and electrolyte levels are relatively stable in both types of sera, but that enzyme levels were more variable in the freeze-dried sera than in the frozen sera.[9] Nonetheless, the freeze-dried sera have some value, because they can be stored economically at room temperatures on a relatively long-term basis and are therefore easier to handle than frozen sera, especially for shipping.

In this report we describe our attempts to validate the use of the frozen and freeze-dried sera for serological and molecular biological detection of HBV and HCV in the AHS longitudinal cohort.

Preparation of frozen and freeze-dried serum samples

The usefulness of stored sera for serological and molecular biological assays of HBV and HCV depends on both preparative methods and storage condition. Therefore, storage methods for serum samples should be optimized for specific purposes; i.e., for epidemiological and clinical studies, for simple screening, etc. It is well documented that HCV RNA is easily degraded by ribonuclease (RNase), which exists in saliva and perspiration.[10] Further, the ability to detect HCV RNA decreases by repeated freezing and thawing of sera.[10] For detection of HBV and HCV, therefore, each serum sample should be dispensed into small volumes at the time of specimen procurement and stored for long-term conservation at –80°C in order to avoid cycles of freezing and thawing of single large samples.

First, we used the following procedure for the preparation of frozen serum samples.[11] Serum was dispensed into four equal volumes and aliquotted into 1.5-ml polypropylene tubes, and stored at –80°C until use. These serum samples were thawed by leaving them at room temperature for 30 minutes and then mixed well by inversion before use.

Second, we used the following procedure for the preparation of freeze-dried serum samples.[11] A 0.4-ml portion of the serum was stored in a glass tube at –80°C, and after one week of storage, these samples were freeze-dried in batches, sealed automatically to maintain a vacuum, and stored at room temperature (20 to 25°C) until needed. The moisture content of freeze-dried serum samples was normally maintained at less than 0.5%, because moisture content of freeze-dried serum samples of less than 1% was necessary to retain and assay various antibodies of interest.[9] These serum samples were reconstituted with the volumetric method using diethyl pyrocarbonate (DEPC)-treated MilliQ water

and mixed well before used.

Serological assays in stored serum samples

To assess the usefulness of stored sera for serological detection of HBV and HCV, we selected at random 12 HBsAg-positive individuals and 25 anti-HCV antibody (Ab)-positive individuals[11] among 6,121 AHS participants who underwent hepatitis screening from 1993 through 1995.[12,13] Their serum samples had been processed and stored by both the freezing and freeze-drying methods.

Detection of HBsAg, anti-HCV Ab, and anti-HBc Ab

The assays of HBsAg and anti-HCV Ab using fresh serum samples were measured by reverse passive hemagglutination (R-PHA) test kit and the second-generation PHA test kit, in hepatitis screening from 1993 through 1995.[12,13] The assays of HBsAg and anti-HCV Ab using frozen and freeze-dried serum samples were measured by enzyme immunoassay (EIA) and the second-generation EIA, respectively.[11]

Comparison of qualitative detection of HBsAg and anti-HCV Ab using fresh, frozen, and freeze-dried serum samples showed good agreement, though the detection methods of HBsAg and anti-HCV Ab in frozen and freeze-dried serum samples were historically different from those in fresh serum samples. The results indicated that freeze-dried sera, as well as frozen sera, are interchangeable with those of fresh sera.[11] This study also showed that freeze-dried sera are interchangeable with frozen sera in the qualitative detection of HBsAg and anti-HCV Ab (Table 1).

Anti-HBc Ab is present during the acute, convalescent, and chronic phases of HBV infection, and persists for many years. Anti-HBc Ab has long been used as supplementary assay to avoid missing HBV carriers such as HBsAg-negative individuals with HBs gene escape mutants or HBsAg-low titer individuals. We did not compare the results of the anti-HBc Ab measured by EIA in frozen and freeze-dried serum samples with those measured by PHA in fresh serum samples, because the sensitivity of the test for anti-HBc Ab is greatly affected by the methodology.[14] Therefore, we compared the EIA results for anti-HBc Ab in frozen serum samples with those in freeze-dried serum samples.[15] Concordance in measurement of anti-HBc Ab among frozen and freeze-dried serum samples was not complete, but was satisfactory. Frozen serum samples of two cases that tested positive for anti-HBc Ab were indeterminant in the freeze-dried serum samples. On the other hand, anti-HBc Ab results of a frozen serum sample of one case proved to be indeterminant, while a freeze-dried serum sample tested negative. For these three cases with discrepant results, the measured values for the anti-HBc Ab specimens that yielded positive or negative result were close to being indeterminant (Table 1).

As a whole, both frozen and freeze-dried sera can be useful for serological assays of HBV and HCV. We can conclude that serological assays using stored sera may be reliable for detection of HBV and HCV. Moreover, use of more sensitive molecular biological assays might well compensate for the loss of serological reactivity in stored sera.

Molecular biological assays in stored serum samples

To assess the usefulness of stored sera for molecular biological detection of HBV and HCV, we also used frozen and freeze-dried serum samples obtained from above-mentioned 12 HBsAg-positive and 25 anti-HCV Ab-positive individuals.[11]

Table 1. Detection of HBsAg, anti-HBc Ab, and anti-HCV Ab by EIA in stored sera

Results of freeze-dried serum samples	Results of frozen serum samples			Concordance (%)
	positive	negative	indeterminant	
HBsAg				
positive	12	0		100
negative	0	25		
Anti-HBc Ab*				
positive	24	0	0	
negative	0	8	1	92
indeterminant	2	0	2	
Anti-HCV Ab				
positive	24	1**		97
negative	0	12		

*Measured values for anti-HBc Ab of ≥70%, <50%, and 50≤ <70% were defined as positive, negative, and indeterminant, respectively.

**Anti-HCV Ab titer is very low and the detection of HCV RNA was negative with highly sensitive qualitative PCR.

Genotyping of HBV and HCV

HBV and HCV genotypes have been recognized as important viral factors related to pathological status, effectiveness of antiviral treatments, and prognosis of liver disease.[1–3,16] Therefore, we have tested the suitability of stored sera for HBV and HCV genotyping.[15] Serum DNA was extracted from either 100 µl of frozen or reconstituted freeze-dried serum samples using QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan), and serum RNA was extracted from 100 µl of frozen or reconstituted freeze-dried serum samples using SepaGene RV-R (Sanko Junyaku Co., Tokyo, Japan). The prepared RNA was reverse transcribed with random primers and reverse transcriptase (ReverTra Ace, TOYOBO Co., Tokyo, Japan). HBV and HCV genotypes were determined by polymerase chain reaction (PCR) using type-specific primers in regions of the pre-S1 through S genes of HBV[17] and the NS5 gene of HCV,[18] respectively.

HBV genotypes could be identified in nine frozen and ten freeze-dried serum samples, all of which were found to be genotype C. HCV genotype 1b was identified in 17 frozen and 16 freeze-dried serum samples. In addition, HCV genotype 2a was identified in each of four frozen and four freeze-dried serum samples tested. (Table 2).

The observed discordance presumably was not due to differences in genotype, but rather due to difficulties in PCR amplification. Since all discordant cases were borderline, or undetectable, in the highly sensitive, qualitative PCR (Table 3), the discordance seemed to be attributable to variations in the detection limits of PCR, arising from the minimal amounts of DNA and RNA. Alternatively, it is possible that the HBV DNA and the HCV RNA had been degraded during prolonged storage of either the frozen or freeze-dried sera. However these findings showed that both frozen and the freeze-dried sera may be useful for HBV and HCV genotyping.

Quantitative detection of HBV DNA and HCV RNA

We compared the efficiencies of HBV-DNA quantification in HBsAg-positive frozen and freeze-dried serum samples. Quantification of HBV DNA was performed using real-time PCR and fluorescence resonance energy transfer probes and primers that were designed for a highly conserved X region.[15] In

Table 2. Genotyping of HBV and HCV in stored sera

Freeze-dried serum samples	No. of frozen serum samples with HBV and HCV genotype results			Concordance (%)
HBV genotype	C	Not detected		
C	9	1		92
Not detected	0	2*		
HCV genotype	1b	2a	Not detected	
1b	16	0	0	
2a	0	4	0	96
Not detected	1	0	4**	

*HBV-DNA concentration was low for detecting some genotypic determinants.
**The detection of HCV RNA was negative with highly sensitive qualitative PCR.

Table 3. Establishment of detection limit for HCV RNA using qualitative PCR assay

HCV RNA (IU/ml)*	Estimated input of HCV RNA (IU)**	No. positive/No. tested (% Positive ratio)
4,700,000	47,000	8/8 (100%)
940,000	9,400	8/8 (100%)
188,000	1,880	8/8 (100%)
37,600	376	8/8 (100%)
7,520	75	8/8 (100%)
1,504	15	8/8 (100%)
301	3	8/8 (100%)
100	1	8/8 (100%)
60	0.6	4/8 (50%)
0	0	0/8 (0%)

*A serum sample known as HCV-RNA concentration by Amplicore HCV monitor test ver. 2.0 (4,700 kIU/ml, genotype 1b) was diluted with serum from an HCV-negative individual.
**Estimated input of HCV RNA in each cDNA solution was applied to the respective qualitative PCR assay.

terms of reproducibility, our analyses showed the intra-assay coefficients of variation (CVs) of 2.4% and 2.5% at two known HBV-DNA concentrations, whereas the inter-assay CVs were 1.4% and 2.5%. Real-time PCR detected HBV DNA in 10 of 12 frozen and freeze-dried serum samples from HBsAg-positive subjects in the 1993–1995 hepatitis screening. The correlation between $\log_{10}$-transformed HBV-DNA quantities for pairs of frozen and freeze-dried serum samples was significant ($r = 0.981$, $P < 0.0001$).

We also compared the efficiencies of HCV-RNA quantification using real-time PCR in anti-HCV-positive frozen and freeze-dried serum samples.[11] In terms of reproducibility, our analyses showed the intra-assay CVs of 6.2% and 2.9% at two known HCV-RNA concentrations, whereas the inter-assay CVs were 3.6% and 4.3%. Real-time PCR detected HCV RNA in 18 of 25 frozen and freeze-dried serum samples from anti-HCV-positive subjects, originally identified in the 1993–1995 hepatitis screening. The correlation between $\log_{10}$-transformed HCV-RNA quantities for pairs of frozen and freeze-dried serum samples was significant ($r = 0.908$, $P < 0.0001$). HCV RNA is easily decomposed by RNase, and its detectability decreases by repeated freezing and thawing of sera. Therefore, it was expected that the PCR products of HCV RNA would vary depending on the method and condition of storage. Nevertheless, the results of our study showed that there were no remarkable differences in our capacity to detect HCV RNA, even when different methodologies and conditions of storage were employed over prolonged periods.

As a whole, both frozen and freeze-dried sera can be useful for molecular biological assays of HBV and HCV. We can conclude that molecular biological assays using stored sera may be reliable for detection of HBV and HCV.

Application of stored sera: A nested case-control study of hepatocellular carcinoma (HCC)

We conducted a nested case-control study of HCC using stored sera in the AHS longitudinal cohort. The study included 224 HCC cases and 644 controls that matched to the cases on gender, age, city, time of serum storage, and method of serum storage (freezing or freeze-drying methods), and counter-matched on radiation dose.[19]

First, HBsAg, anti-HBc Ab, anti-HCV Ab, and HCV RNA were measured as viral factors using available stored sera of 211 cases and 640 controls obtained before the HCC diagnosis. Qualitative detection of HCV RNA was performed by PCR using two sets of primers corresponding to the 5′-untranslated region.[20] The detection limit was established by limiting dilution method.[15] This highly sensitive qualitative PCR assay detected as HCV-RNA positive in 100% of serum samples containing 100 IU/ml of HCV RNA, and 50% of the samples containing 60 IU/ml of HCV RNA (Table 3). HBV infection status was defined as positive by the detection of HBsAg, or having a high titer of anti-HBc Ab. HCV infection status was defined as positive by the detection of HCV RNA.

Among HCC cases, 13.7% were found to be HBV only infections, 62.6% were HCV only infections, and 2.4% were positive for both; while the remaining subjects negative for both. Among the controls, 2.8% were found to be HBV only infections, 6.4% were HCV only infections, and 0.3% were positive for both. These distributions of HBV and HCV infection status were similar to those in previous reports on Japanese populations.[21]

Conclusions

The identification of HBV and HCV infections in participants of AHS cohort can be accomplished using both frozen and freeze-dried sera, and this identification can be accurate and reliable depending on the method, condition and duration of storage, and associated optimizations. Therefore, it is expected that the sera stored for long periods will be useful for future viral hepatitis studies, e.g., evolution of the hepatitis virus as well as the natural history of viral liver diseases.

References

1. Orito E, Mizokami M, Sakugawa H, Michitaka K, Ishikawa K, Ichida T, Okanoue T, Yotsuyanagi H, Iino S. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. *Hepatology* 33:218-23, 2001.

2. Wai CT, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 36:1425-30, 2002.

3. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. Mutations in the nonstructual protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 334:77-81, 1996.

4. Chayama K, Tsubota A, Kobayashi M, Okamoto K, Hashimoto M, Miyano Y, Koike H, Kobayashi M, Koida I, Arase Y, Saitoh S, Suzuki Y, Murashima N, Ikeda K, Kumada H. Pretreatment virus load and multiple amino

acid substitutions in the interferon sensitivity-determining region predict the outcome of interferon treatment in patients with chronic genotype 1b hepatitis C virus infection. *Hepatology* 25:745-9, 1997.

5. Mendy M, Kirk GD, van der Sande M, Jeng-Barry A, Lesi OA, Hainaut P, Sam O, McConkey S, Whittle H. Hepatitis B surface antigenaemia and alpha-foetoprotein detection from dried blood spots: Applications to field-based studies and to clinical care in hepatitis B virus endemic areas. *J Viral Hepat* 12:642-7, 2005.

6. Seeff LB, Miller RN, Rabkin CS, Buskell-Bales Z, Straley-Eason KD, Smoak BL, Johnson LD, Lee SR, Kaplan EL. 45-year follow-up of hepatitis C virus infection in healthy young adults. *Ann Intern Med* 132:105-11, 2000.

7. Das PC, de Vries AH, McShine RL, Sibinga CT. Dried sera for confirming blood-borne virus infections (HCV, HTLV-I, HIV & HBsAg). *Transfus Med* 6:319-23, 1996.

8. Jardi R, Rodriguez-Frias F, Buti M, Schaper M, Valdes A, Martinez M, Esteban R, Guardia J. Usefulness of dried blood samples for quantification and molecular characterization of HBV-DNA. *Hepatology* 40:133-9, 2004.

9. Radiation Effects Research Foundation. Symposium on serum storage methods. Hiroshima Igaku [*J Hiroshima Med Assoc*] 42:1596-609, 1989. (Japanese)

10. Halfon P, Khiri H, Gerolami V, Bourliere M, Feryn JM, Reynier P, Gauthier A, Cartouzou G. Impact of various handling and storage conditions on quantitative detection of hepatitis C virus RNA. *J Hepatol* 25:307-11, 1996.

11. Ohishi W, Fujiwara S, Suzuki G, Kishi T, Sora M, Matsuura S, Hakoda M, Tatsukawa Y, Yamada M, Chayama K. Feasibility of freeze-dried sera for serological and molecular biological detection of hepatitis B and C viruses. *J Clin Microbiol* 44:4593-5, 2006.

12. Fujiwara S, Kusumi S, Cologne J, Akahoshi M, Kodama K, Yoshizawa H. Prevalence of anti-hepatitis C virus antibody and chronic liver disease among atomic bomb survivors. *Radiat Res* 154:12-9, 2000.

13. Fujiwara S, Sharp GB, Cologne JB, Kusumi S, Akahoshi M, Kodama K, Suzuki G, Yoshizawa H. Prevalence of hepatitis B virus infection among atomic bomb survivors. *Radiat Res* 159:780-6, 2003.

14. Ohishi W, Mori N, Tsuge M, et al. Examination about the clinical usefulness of anti-HBc Ab measurement by Architect. *Acta Hepatol Jpn* 45: 555-6, 2004. (Japanese)

15. Ohishi W, Fujiwara S, Suzuki G, Chayama K. Validation of the use of freeze-dried sera for the diagnosis of hepatitis B and C virus infections in a longitudinal study cohort. Mohan RM, ed. *Research Advances in Microbiology 7*. Global Research Network, Kerala, India, 2007. pp. 1-9.

16. Orito E, Ichida T, Sakugawa H, Sata M, Horiike N, Hino K, Okita K, Okanoue T, Iino S, Tanaka E, Suzuki K, Watanabe H, Hige S, Mizokami M. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* 34:590-4, 2001.

17. Naito H, Hayashi S, Abe K. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. *J Clin Microbiol* 39:362-4, 2001.

18. Chayama K. Genotyping hepatitis C virus by type-specific primers for PCR based on NS5 region. Lau JYN, ed. *Methods in Molecular Medicine, vol. 19: Hepatitis C Protocols*. pp 165-73, Humana Press Inc., Totowa, NJ, 1998.

19. Cologne JB, Sharp GB, Neriishi K, Verkasalo PK, Land CE, Nakachi K. Improving the efficiency of nested case-control studies of interaction by selecting controls using counter matching on exposure. *Int J Epidemiol* 33:485-92, 2004.

20. Arataki K, Kumada H, Toyota K, Ohishi W, Takahashi S, Tazuma S, Chayama K. Evolution of hepatitis C virus quasispecies during ribavirin and interferon-alpha-2b combination therapy and interferon-alpha-2b monotherapy. *Intervirology* 49:352-61, 2006.

21. Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, Tanaka E. Hepatocellular carcinoma: Recent trends in Japan. *Gastroenterology* 127:S17-26, 2004.

Molecular Features of Colorectal Cancer Developing among Atomic Bomb Survivors with Emphasis on Microsatellite Instability – An Interim Report –

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Introduction

More than sixty years have passed since the atomic bombings, but the excess relative risk (ERR) of all solid cancers remains high. As indicated in a recent follow up report on the Life Span Study (LSS) of atomic-bomb (A-bomb) survivors, the effect of radiation exposure on the risk of solid cancer varies, sometimes appreciably, for different bodily organs.[1] Colon cancer has a high ERR per Sv of 0.72 (95% confidence interval, 0.29–1.3) for radiation exposure. Rectal cancer, by contrast, has a relatively low ERR. The biologic basis of such differences in exposure risks are unclear. Likewise, the molecular mechanisms by which radiation induces solid cancers, especially colon cancer, are ill defined.

Apart from colorectal cancers among A-bomb survivors, development of such cancers after radiotherapy was first reported in 1957.[2] Accumulated information since then revealed that radiotherapy-induced colorectal cancer showed a high frequency of cancers with mucinous/poorly differentiated histology.[3,4]

Two major molecular events have been tied to colorectal carcinogenesis, namely chromosomal instability and microsatellite instability (MSI). High-MSI (MSI-H) is correlated with mucinous/poorly differentiated histology and a specific sub site of the colon—frequently the proximal colon.[5] We hypothesized therefore, that the MSI phenotype may be associated with radiation exposure in colorectal cancer patients of the A-bomb survivor cohort.

Most MSI-H sporadic colorectal cancer cases showed a loss of DNA repair enzyme MLH1 expression. Methylation of the *MLH1* gene promoter is thought to be a major cause for inactivation of the *MLH1* gene in sporadic cancer.[6] Importantly, methylation of CpG dinucleotides within only small regions of *MLH1* gene promoter at its proximal region (–248 to –178 bp) has been shown to be closely associated with lowered expression of *MLH1* mRNA[7,8] and protein.[9] In addition to DNA methylation, loss-of-heterozygosity (LOH) of the *MLH1* gene also contributes to *MLH1* gene defects in some sporadic colorectal cancer with MSI.[6]

During the past decade, a "serrated polyp pathway" has been identified and shown to be mechanistically important in terms of colon cancer. This pathway is initiated with development of hyperplastic polyp in right-sided colon, and is followed by serrated adenoma and invasive carcinoma. Notably, this pathway is associated with MSI.[10] In the serrated polyp pathway, loss of MLH1 protein followed by acquisition of MSI has been shown to occur in the late stages.[11] The constitutive activation of the RAS-RAF-MEK-ERK-MAP kinase-signaling pathway, which is caused by gene mutations of mainly *BRAF* and to a lesser extent *KRAS*, is recognized as an early event of colon tumorigenic process(es) following the serrated polyp pathway.[12] In addition to these mutations, the lack of RASSF2 function (a modulator of apoptosis signal that is dependent upon binding to K-Ras) that is due to methylation of its gene promoter, has been recently identified as an important activator of the signaling pathway in colorectal carcinogenesis.[13,14,15]

In this article, we present an interim report on our molecular oncology study and the MSI phenotype of colorectal cancer in A-bomb survivors.

Materials and Methods

Study subjects

For molecular analysis of colorectal cancer cases among A-bomb survivors, 35 study materials of formalin-fixed and paraffin-embedded tissue specimens

of subjects that had undergone surgery or endoscopy at medical institutions in Hiroshima during 1986–2001 with clinico-pathological data were obtained and unlinkably anonymized through the Study Group on Atomic Bomb Diseases, entrusted by the Ministry of Health, Labour and Welfare (MHLW), Japan.

This study was conducted under approval of the Genetic and Medical Ethics Commission at Hiroshima University, and the Human Investigation Committee and the Ethics Committee for Genome Research at the Radiation Effects Research Foundation (RERF). A-bomb radiation doses used in this analysis were estimated by the recently implemented DS02 system.[16]

DNA preparation

Using hematoxylin and eosin stained tissue sections, a pathologist (RI) performed pathological reviews. Two cases did not provide sufficient amounts of non-cancerous tissue. Five-µm tissue sections were deparaffinized with Hemo-De (Falma, Tokyo, Japan), stained with Methyl Green (Sigma-Aldrich, St. Louis, MO) and dissected manually or using laser microdissection system AS LMD (Leica, Wetzler, Germany). DNA was extracted from the microdissected non-cancerous or cancerous regions using QIAamp DNA Micro Kit (Qiagen, Hilden, Germany).

Detection of microsatellite instability

To determine the extent of MSI, five microsatellite markers (*BAT25*, *BAT26*, *D2S123*, *D5S346,* and *D17S250*) recommended by a National Cancer Institute (NCI) workshop on MSI[17] were examined. Another mononucleotide repeat marker *NR-21*[18] was examined as well, following a recent recommendation by another NCI workshop on MSI.[19] Polymerase chain reaction (PCR) was performed in a 20-µl mixture containing 5 pmoles of unlabeled primer, 5 pmoles of fluorescently-labeled primer, 250 µM of each dNTP, 0.5 U of AccuSure DNA polymerase (Bioline Ltd., London, UK), 10 ng of genomic DNA and 1× reaction buffer supplied by the manufacturer. PCR conditions consisted of initial denaturation (95°C for 10 min), followed by 60 cycles (denaturation at 95°C for 15 sec, annealing for 15 sec, extension at 72°C for 15 sec). Primer sequences and annealing temperatures are summarized in Supplementary Table 1. The fluorescently labeled amplicons with CEQ DNA Size Standard Kit-400 (Beckman

Table 1. Clinico-pathological and epidemiological characteristics of the study subjects

		This study	LSS*	LSS**	*P*†
Gender	Male (n)	13			
	Female (n)	22			
Organ	Colon (n)	24	457		0.2
	Rectum (n)	11	351		
Location	Proximal (n)	11			
	Distal (n)	24			
Histology	Well (n)	10		80	0.5
	Moderately (n)	18		105	
	Poorly/Mucinous (n)	7		30	
Age at operation	Mean (yrs)	73.3			
	SD	9.4			
	Range (yrs)	53, 91			
Age at A-bombing	Mean (yrs)	25.2			
	SD	10.8			
	Range (yrs)	3, 48			
Time since A-bombing	Mean (yrs)	48.1			
	SD	4.6			
	Range	41, 56			
Radiation dose	Median (mGy)	26			
	Range	1, 2140			
	Interquartile range	128			

*Data obtained from a LSS report of cancer incidence (1958–1987) by Thompson DE *et al.*[1]
**Data obtained from a pathological report of colorectal cancer cases among A-bomb survivors (1955-1980) Nakatsuka H *et al.*[32]
† Chi-square test.

Coulter Inc., Fullerton, CA) were subjected to capillary-electrophoresis using DNA sequencer CEQ8000 (Beckman Coulter). Microsatellites were judged to be unstable if one or more novel bands were present in the amplicons of the tumor samples compared with non-cancerous samples of the same subjects. A tumor was considered to be MSI-H if 40% or more of the amplified markers were unstable, MSI-L if fewer than 40% of the markers were unstable, and MSS if none of the markers was unstable.

Detection of gene mutations of BRAF and KRAS genes

BRAF gene mutation-causing amino acid substitution of glutamic acid for valine at codon 600 (*BRAF*^{V600E}) was determined by restriction fragment length polymorphism (RFLP) using *TspR* I (New England Biolabs, Ipswich, MA) and direct sequencing, as described previously.[20] Exon 2 region of the *KRAS* gene was amplified by PCR. PCR was performed in a 20-µl mixture containing 5 pmoles each of primer, 250 µM of each dNTP, 0.5 U of AccuSure DNA polymerase (Bioline Ltd.), 10 ng of genomic DNA, and 1× reaction buffer supplied by the manufacturer. PCR conditions consisted of initial denaturation (95°C for 10 min), followed by rounds of cycles (denaturation at 95°C for 15 sec, annealing for 15 sec, extension at 72°C for 15 sec). First round PCR was conducted for 60 cycles using primers KR12-4Fa: 5′-CCT GCT GAA AAT GAC TGA ATA TAA-3′ and KR12-2R: 5′-AAT GAT TCT GAA TTA GCT GTA TCG-3′. The first PCR product was diluted to 1/1,000 using MilliQ water and subjected to nested PCR of 25 cycles using primers M13M3KRASF001: 5′-GTA AAA CGA CGG CCA GTT GAA AAT GAC TGA ATA TAA ACT TGT G-3′, and M13RVNKRASR001: 5′-TGT GGA ATT GTG AGC GGG CTG TAT CGT CAA GGC ACT C-3′, with the underlined sequences derived from an M13 vector. The PCR products were purified using MinElute PCR Purification Kit (Qiagen). Sequencing of the purified fragments was performed using CEQ Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit (Beckman Coulter Inc., Fullerton, CA), M13priM3: 5′-GTA AAA CGA CGG CCA GT-3′ or M13RVN: 5′-TGT GGA ATT GTG AGC GG-3′ as a primer, and DNA sequencer CEQ8000 (Beckman Coulter).

Analysis of DNA methylation of MLH1 and RASSF2 genes

Methylation of the *MLH1* and *RASSF2* genes was carried out using combined bisulfite restriction analysis (COBRA).[21] Two hundred ng of DNA was treated with bisulfite and purified using EZ DNA Methylation Kit (ZYMO RESEARCH, Orange, CA). Ten ng of the bisulfite-converted DNA was subjected to semi-nested PCR. PCR was performed in a 20-µl mixture containing 5 pmoles of each primer, 250 µM of each dNTP, 0.5 U of AccuSure DNA polymerase (Bioline Ltd.), 10 ng of bisulfite-converted DNA, and 1× reaction buffer supplied by the manufacturer. PCR conditions consisted of initial denaturation (95°C for 10 min), followed by cycles (denaturation at 95°C for 15 sec, annealing for 15 sec, extension at 72°C for 15 sec), with 55 and 30 cycles for the first and second rounds of PCR, respectively. For *MLH1*, the first round of PCR was conducted using primers MehMLH1F7: 5′-AAA AAY GAA TTA ATA GGA AGA GYG GAT-3′ and MehMLH1R12: 5′-CCT AAA ACR ACT ACT ACC CRC TAC CTA A-3′, followed by a second round of PCR using primers MehMLH1F7 and MehMLH1R11: 5′-CTA AAA CRA CTA CTA CCC RCT ACC TAA AAA-3′. For *RASSF2*, the first round of PCR was conducted using primers, MeRASSF2F1: 5′-GGY GGG AGT TTG GAY GAG T-3′ and MeRASSF2R1: 5′-CCT CCC TCC CAA ACA ATA CTA-3′, followed by a second round of PCR using primers MeRASSF2F1 and MeRASSF2R2: 5′-TCC CTC CCA AAC AAT ACT ATA ACT A-3′. All the primers used for this methylation analysis were designed with MethPrimer.[22] The resultant PCR products were digested with restriction enzyme *Mlu* I (Fermentas, Vilnius, Lithuania) for *MLH1* and *Hpy*CH4 IV (New England Biolabs) for *RASSF2*. The undigested and digested PCR products were electrophoresed on 12% polyacrylamide gel. After staining with SYBR Gold nucleic acid gel stain (Invitrogen, Carlsbad, CA), the image was visualized under ultraviolet (UV) illumination using ChemImager 5500 (Alpha Innotech, San Leandro, CA).

Analysis of LOH at DNA mismatch repair enzyme genes MLH1 and MSH2

LOH in the *MLH1* and *MSH2* genes was evaluated with three methods based on either microsatellite markers or single nucleotide polymorphisms (SNP). Three microsatellite markers, *D3S1611*, *D3S1007,* and *D3S1561,* were used for *MLH1*, while *D2S378*, *D2S391,* and *D2S136* were examined for *MSH2*. Primer sets and annealing temperatures are summarized in Supplementary Table 2. PCR reaction and auto-sequencer detection were the same as those described for MSI detection.

DNA fragments encompassing SNP loci rs9857252, rs1800734, and rs4535177 located 5′ to the *MLH1* gene and rs1981928, rs2347794, rs3771274, rs6726691, and rs2042649 within the *MSH2* genes were amplified. Detection of SNP was conducted with RFLP except for rs4535177. Primer sets, annealing temperatures, and restriction enzymes are shown in Supplementary Table 3. After digestion of restriction enzymes that are sensitive for the SNP, the resultant fragments were electrophoresed

Table 2. Association of MSI status with clinico-pathological and epidemiological actors

		MSS (n=15)	MSI-L (n=13)	MSS/MSI-L (n=28)	MSI-H (n=5)	*P*
Gender	Male (n)	8	5	13	0	0.1*
	Female (n)	7	8	15	5	
Organ	Colon (n)	9	8	17	5	0.1*
	Rectum (n)	6	5	11	0	
Location	Proximal (n)	4	2	6	5	0.002*
	Distal (n)	11	11	22	0	
Histology	Well (n)	5	5	10	0	0.004**
	Moderately (n)	9	6	15	1	
	Poorly/Mucinous (n)	1	2	3	4	
Age at A-bombing	Mean (yrs, SD)	72.7 (9.0)	73.2 (10.9)	73.0 (9.7)	71.0 (5.3)	0.7†
Age at operation	Mean (yrs, SD)	23.7 (10.2)	25.8 (12.1)	24.6 (11.0)	21.0 (4.7)	0.5†
Time since A-bombing	Mean (yrs, SD)	49.1 (4.5)	47.5 (4.2)	48.3 (4.4)	48.2 (6.3)	1.0†

*Fisher's exact test for MSS/MSI-L vs. MSI-H.
**Mann-Whitney's U test for MSS/MSI-L vs. MSI-H. †Student's t test.

Table 3. Association of *MLH1* gene methylation with clinico-pathological and epidemiological factors and MSI status

		MLH1		
		Unmethylated (n=30)	Methylated (n=5)	*P*
Gender	Male (n)	12	1	0.6*
	Female (n)	18	4	
Organ	Colon (n)	20	4	1.0*
	Rectum (n)	10	1	
Location	Proximal (n)	7	4	0.026*
	Distal (n)	23	1	
Histology	Well (n)	10	0	0.031**
	Moderately (n)	16	2	
	Poor/Mucinous (n)	4	3	
Age at A-bombing	Mean (yrs, SD)	74.0 (9.8)	69.6 (5.8)	0.3†
Age at Operation	Mean (yrs, SD)	25.7 (11.3)	22.2 (7.1)	0.5†
Time since A-bombing	Mean (yrs, SD)	48.2 (4.6)	47.4 (5.3)	0.7†
MSI	MSS/MSI-L (n)	26	2	0.017*
	MSI-H (n)	2	3	

*Fisher's exact test.
**Mann-Whitney's U test.
† Student's t test.

on 12% polyacrylamide gel. Restriction enzymes *Hha* I, *Pvu* II, *Hpy*CH4 V, and *Hind* III were purchased from New England Biolabs and *Tas* I was from Fermentas. SNP information was obtained from the National Center for Biotechnology Information (NCBI).

For rs4535177, the tetra-primer amplification refractory mutation system (ARMS) method was applied for polymorphism detection.[23] A first round of PCR was conducted in a 20-µl mixture containing 10 ng of DNA, 5 pmoles each of primer Rs4535177OF1: 5′-GAT TAT TTC ACT GGT GGT

ATT GGA TT-3′ and Rs4535177OR1: 5′-ACC TTT TCC CTT TGT TTT CCC-3′, 250 μM of each dNTP, 0.5 U of AccuSure DNA polymerase, and 1× reaction buffer. PCR conditions consisted of initial denaturation (95°C for 10 min), followed by 65 cycles (denaturation at 95°C for 30 sec, 60° for 30 sec, extension at 72°C for 30 sec). After dilution of the first PCR product 1,000 fold with MilliQ water, a second PCR was conducted using 1.5 pmoles each of primers Rs4535177OF1 and Rs4535177OR1, 15 pmoles of Rs4535177IF1: 5′-GGA TTT CTG GCC ATT TCA CA-3′, and 100 pmoles of Rs4535177IR1: 5′-ACA AAA CCA CCT GGC TTG AC-3′. PCR conditions consisted of initial denaturation (95°C for 10 min), followed by 5 cycles (denaturation at 95°C for 30 sec, 65°C for 30 sec, extension at 72°C for 30 sec with decreasing annealing temperature 1°C per cycle), and 30 cycles (denaturation at 95°C for 30 sec, 60°C for 30 sec, extension at 72°C for 30 sec). The resultant PCR products were electrophoresed on 12% polyacrylamide gel.

Statistical analyses

We compared continuous variables using Student's t-test. The difference of proportions in contingency tables was evaluated by Fisher's exact test or chi-square test. The Mann-Whitney U test was used for non-parametrical comparison of continuous and categorical variables between two groups. The Jonckheere-Terpstra test was used for assessing non-parametric trends of continuous variables among three groups. Statistical analyses were conducted with the use of SPSS software (ver. 12.0J) and the R language (a language and environment for statistical computing, ver. 2.3.1).[24]

Results

Clinico-pathological and epidemiological characteristics of colorectal cancer patients among A-bomb survivors

Clinico-pathological and epidemiological characteristics of study subjects are shown in Table 1. The frequency of mucinous/poorly differentiated cancer (20.0%) was slightly higher than that of a previous report of colorectal cancer among LSS participants who were diagnosed between 1950–1980 (13.6%), though the difference was not statistically significant. The mean time that had elapsed since the atomic-bombings (A-bombings) was 48.1 years.

Association of MSI and radiation exposure

At first, we determined MSI status of 33 colorectal cancers from A-bomb survivors with both cancerous and non-cancerous tissue available. Represen-

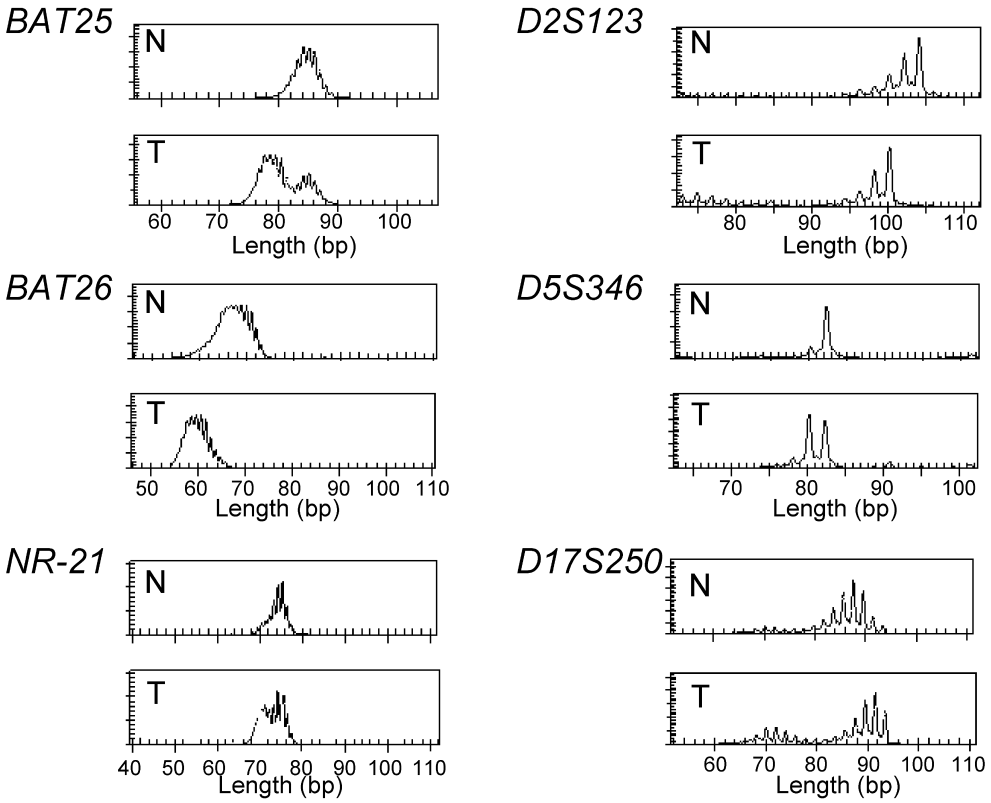

Figure 1. Electrograms of microsatellite markers showing MSI. Representative patterns of electrograms are shown for fluorescently labeled amplicons of DNA samples from tumor and normal tissue. N, normal; T, tumor.

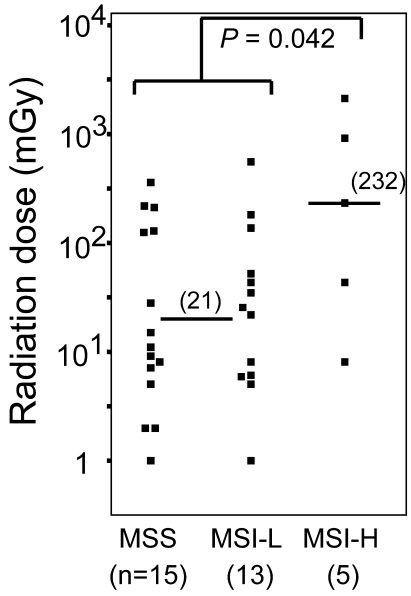

Figure 2. Association between radiation dose and MSI status. Distribution of radiation dose for each patient was plotted by MSI status. Numbers in parentheses indicate median values. Mann-Whitney U test was conducted for MSS/MSI-L vs. MSI-H.

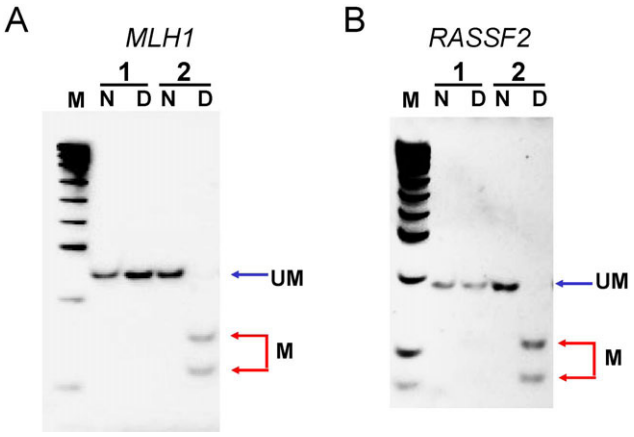

Figure 3. Methylation analysis of *MLH1* and *RASSF2* genes by COBRA. COBRA was carried out using bisulfite-treated DNA. M, methylated; UM, unmethylated. Representative results are shown. Sample #1 did not show methylation, while sample #2 demonstrated methylation. (A) Methylation analysis of *MLH1* gene. N, not digested; D, digested with restriction enzyme *Mlu* I. (B) Methylation analysis of *RASSF2* gene. N, not digested; D, digested with restriction enzyme *Hpy*CH4 IV.

tative results of analysis of microsatellite markers showing MSI are shown in Figure 1. As a result, five MSI-H cases were observed and all of them were female (Table 2). MSI status was significantly associated with proximal-location and mucinous/poorly differentiated histology ($P = 0.002$ and $P = 0.004$, respectively, Table 2), in good agreement with a previous report on sporadic colorectal cancer.[25] Notably, the median radiation dose of MSI-H colorectal cancer cases was significantly higher than that of MSS/MSI-L cases (232 vs. 21 mGy, $P = 0.042$, Mann-Whitney U test; Figure 2), suggesting a possible association between radiation exposure and MSI.

Gene alterations responsible for MSI

To explore the molecular events responsible for MSI, we first examined methylation of CpG dinucleotides of the *MLH1* gene at the proximal region (–231 to –228 bp). Representative results of COBRA for *MLH1* gene methylation are shown in Figure 3A. Methylation status of the *MLH1* gene was determined in five cases, and was shown to be significantly associated with location and histology ($P = 0.026$ and $P = 0.031$, respectively, Table 3). Furthermore and importantly, the median radiation dose of cases with *MLH1* methylation tended to be higher than that of *MLH1*-unmethylated cases (134 vs. 19 mGy, $P = 0.059$; Figure 4). Gene methylation was also associated with MSI status in this study ($P = 0.017$, Table 3), with three cases showing both methylation of the *MLH1* gene and MSI-H phenotype.

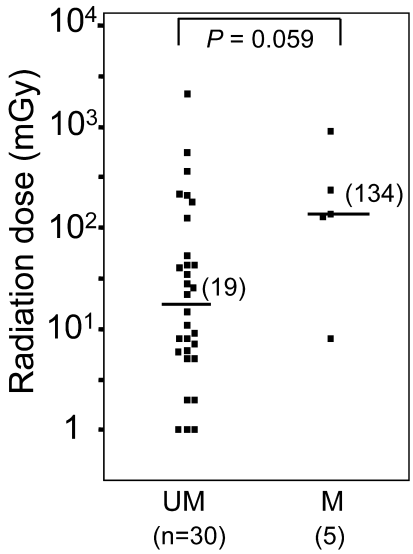

Figure 4. Association between radiation dose and *MLH1* gene methylation. Distribution of radiation dose for each patient was plotted by methylation status. M, methylated; UM, unmethylated. Numbers in parentheses indicate median values. Mann-Whitney U test was conducted for UM vs. M.

In addition to DNA methylation, we also exam-

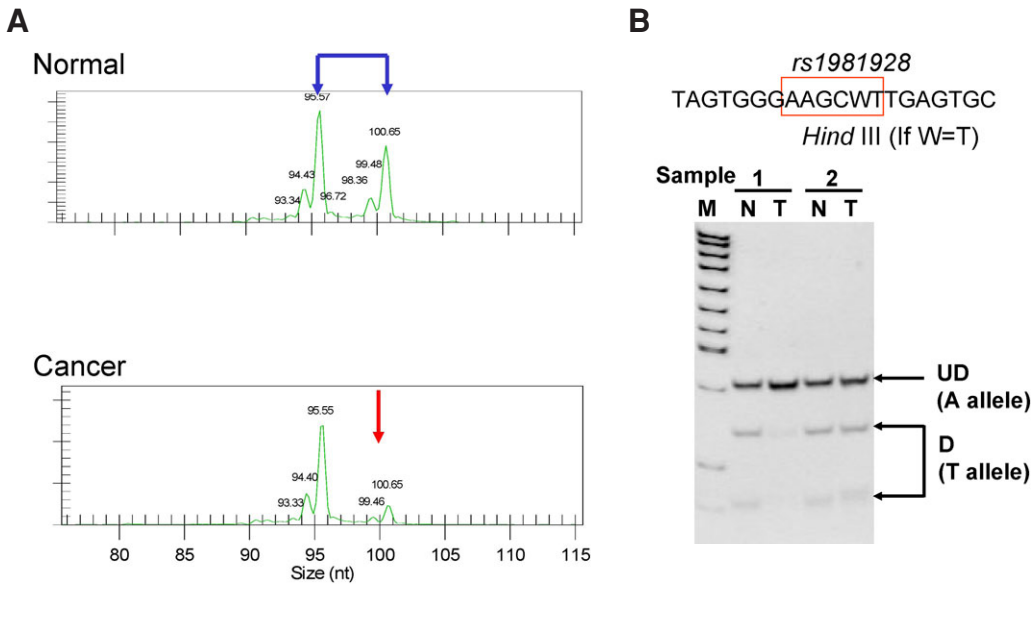

Figure 5. Detection of LOH. (A) Detection of LOH was performed using microsatellite markers. Representative patterns of electrograms are shown for fluorescently labeled amplicons of DNA samples from cancer and normal tissue from a patient. Vertical blue arrows indicate the position of two alleles, while a red arrow indicates the loss of an allele. (B) Detection of LOH was performed using RFLP. The upper part of the figure shows the name of the SNPID along with the sequence and the restriction enzyme used for detection. W indicates A/T heterozygote. The lower figure demonstrates representative eloctrophoresis of samples. N, not treated; T, treated with *Hind* III; D, digested; UD, undigested. (C) Detection of LOH was performed using tetra-primer ARMS method. Horizontal bars indicate the positions of fragments generated by outer primers, G allele specific fragment and A allele specific fragment, respectively.

ined LOH at the gene loci of *MLH1* and *MSH2*. Representative results of LOH detection are shown in Figure 5. We found that all five cases showing MSI-H demonstrated LOH at *MLH1*, while only one case showed LOH at the *MSH2* gene loci (Table 4). These data indicate that development of MSI among A-bomb survivors may mainly be due to both epigenetic and genetic alterations of the *MLH1* gene.

Table 4. LOH and methylation of DNA repair genes in 5 MSI-colorectal cancer cases

Case	*MLH1* methylation	*MLH1* LOH	*MSH2* LOH
A	Methylated	Loss	Loss
B	Methylated	Loss	Retained
C	Methylated	Loss	Retained
D	Unmethylated	Loss	Retained
E	Unmethylated	Loss	Retained

Gene alterations relative to Ras-signaling

To examine whether radiation exposure is associated with MSI-related early molecular events, we next analyzed mutations of *BRAF* at codon 600 and the *KRAS* gene at codons 12/13, as well as methylation of the *RASSF2* gene. Representative results of detecting *RASSF2* gene methylation are shown in Figure 3B, while those results of *KRAS* gene mutations at codons 12/13 are shown in Figure 6. Frequency and types of *KRAS* mutations are summarized in Table 5, along with data of colorectal cancer from a general Japanese population.[26] In brief, no significant difference of mutation type distribution was observed.

Results of methylation of the *RASSF2* gene and mutations of the *KRAS* and $BRAF^{V600E}$ genes in 35 colorectal cancer cases are summarized in Table 6. Although mutual exclusiveness between *KRAS* and

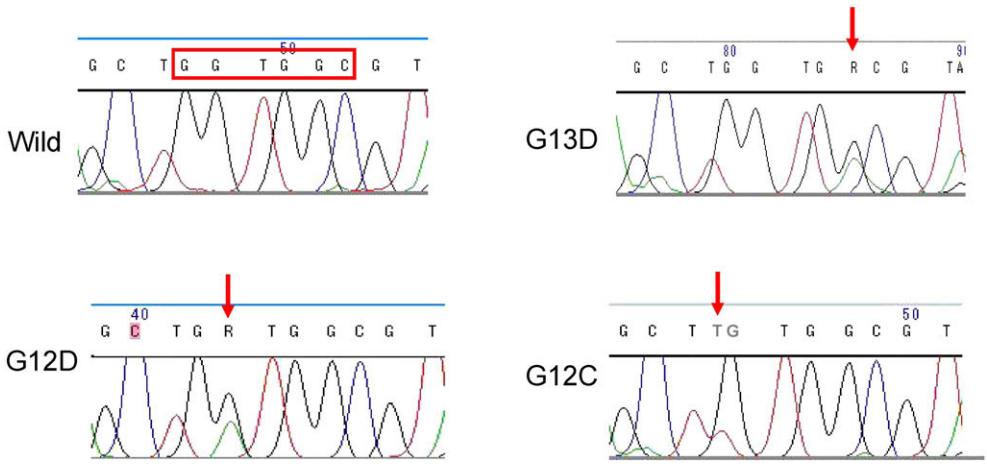

Figure 6. Detection of *KRAS* gene mutations. Direct sequencing of PCR fragments encompassing codons 12 and 13 was conducted. Representative results are shown. G12D, substitution of Gly for Asp at codon 12; G13D, Gly for Asp at codon 13; G12C, Gly for Cys at codon12. Vertical arrows indicate position of mutated nucleotides.

BRAF^{V600E} mutation-based cancers has repeatedly been reported in colorectal cancer by several groups,[27,28,29,30] we found one case that possessed both *KRAS* and *BRAF*^{V600E} mutations simultaneously among a total of 35 examined cases (Table 6).

We subsequently explored the association between *KRAS and BRAF*^{V600E} gene alterations and radiation dose. First, the median radiation dose of the colorectal cancer cases that bore any of these gene alterations was significantly higher than the median radiation dose of cases lacking these alterations (86 vs. 9 mGy, $P = 0.041$; Figure 7A). In addition, we found a significant increasing trend of radiation dose with increased number of gene alterations ($P = 0.016$, Figure 7B).

This Ras-signal alteration status was also found to be closely related to not only MSI but also *MLH1* gene methylation ($P = 0.044$, and $P = 0.045$, respectively; Table 7). In fact, all MSI-H and *MLH1*-methylated cases were found to carry at least one of these alterations. These data imply that radiation exposure may affect early molecular events in the colorectal carcinogenetic pathway relative to MSI among A-bomb survivors.

Discussion

In this study, we found for the first time association between MSI-H phenotype and higher radiation dose in colorectal cancer patients among A-bomb survivors (1986–2001) (Figure 2). Association between MSI-H phenotype and mucinous/poorly differentiated histology, which is commonly observed sporadic colorectal cancer,[5] was also found in our study subjects (Table 2). Interestingly, the frequency of mucinous/poorly differentiated cancer (7/35, 20%) in this study was slightly higher (13.6%) than that value previously reported in a LSS pathology study (1950–1980), and significantly higher, albeit marginally so, than that reported for the general Japanese population (83/764, 10.9%).[31] The

Table 5. Comparison of frequencies of *KRAS* gene mutation types between colorectal cancer patients from A-bomb survivors and general Japanese population

Substitutions		This study		General Japanese*		
Amino acid	Nucleotide	(n)	(%)	(n)	(%)	*P***
G12D	G35A	4	11.4	30	12.7	0.8
G12V	G35T	1	2.9	15	6.4	
G12S	G34A	0	0	5	2.1	
G12A	G35C	1	2.9	4	1.7	
G12C	G34T	1	2.9	2	1.2	
G13D	G38A	1	2.9	12	5.1	
Others	Others	0	0	4	1.7	
No mutation		27	77	162	69.1	
Total		35	100	234	100	

*Data obtained from a report by Nagasaka T *et al.*[26]
**Fisher's exact test for 2 × 8 table.

Table 6. Summary of gene alterations relative to Ras-signaling in 35 colorectal cancers among A-bomb survivors

		RASSF2						
		Unmethylated			Methylated			
		BRAF^{V600E}			*BRAF*^{V600E}			
		Wild	Mutated	Total	Wild	Mutated	Total	Total
KRAS	Wild (n)	17	1	18	7	2	9	27
	Mutated (n)	6	1	7	1	0	1	8
Total (n)		23	2	25	8	2	10	35

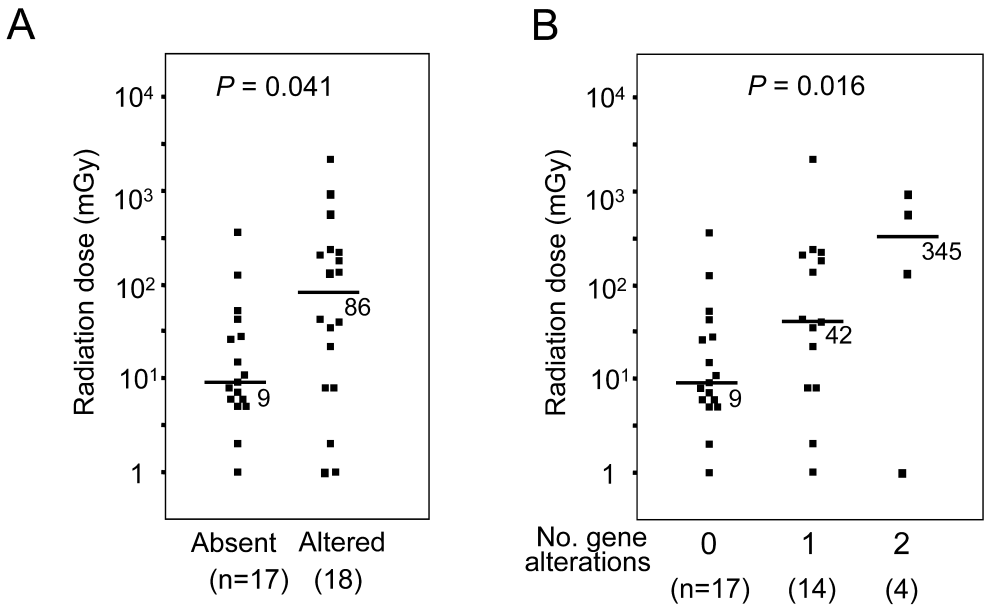

Figure 7. Association between gene alterations relative to Ras-signaling and radiation dose. (A) Distribution of radiation dose for each patient was plotted by the presence or absence of gene alterations. Numbers in parentheses indicate median values. Mann-Whitney's U test was conducted for absent vs. present. (B) Distribution of radiation dose for each patient was plotted by the numbers of gene alterations. Jonckheere-Terpstra test was conducted for assessing increasing trend of radiation dose among the three groups (one sided).

Table 7. Association of ras-signaling alterations and *MLH1* gene methylation or MSI status

		Ras signaling alterations†			
		Absence	Presence	P_{trend}	*P*
MLH1 methylation	UM (n)	17	13		0.045**
	M (n)	0	5		
MSI	MSS (n)	9	6	0.040*	0.044**
	MSI-L (n)	7	6		
	MSI-H (n)	0	5		

† Ras signaling alterations were assessed by mutations of *BRAF*^{V600E} and *KRAS* gene codons 12 and 13, and methylation of *RASSF2* gene.
*Cochran-Armitage test,
**Fisher's exact test for MSS/MSI-L vs. MSI-H.

latter finding is in contrast to a previously reported pathology study on colorectal cancer among A-bomb survivors who were diagnosed between 1955 and 1980, and the absence of a discernable association between histology and radiation dose.[32]

A higher frequency of mucinous/poorly differentiated cancer among radiation-associated cancers has also been reported for colorectal cancer after pelvic irradiation.[4] The time since radiotherapy for these subjects appeared to be distributed uniformly from five to over 40 years, with a median time of 20 years; the median time since radiotherapy for development of colorectal cancer with mucinous/poorly differentiated histology was significantly longer than that for well/moderately differentiated (27 vs. 20 yrs).[4] Since the percentage of cancer cases with mucinous/poorly differentiated histology did not change by age at diagnosis group among the Japanese general population,[31] the observed increase of time since radiotherapy for mucinous/poorly differentiated cancer may not be due to the late-onset of cancer with these histological types, but rather due to the characteristic features of radiation-induced cancer. Besides the development of second primary colorectal cancer following radiotherapy, there was a report of radiation-associated cancers after radiotherapy of healthy individuals: three healthy Jewish teenagers in the Auschwitz concentration camp during World War II were exposed to a single massive dose of irradiation as part of sterilization experiments, and they contracted colorectal cancer 40 years after the irradiation.[33]

We observed that radiation exposure was associated with gene alterations relative to Ras-signaling in colorectal cancer among A-bomb survivors (Figure 7). This suggests that acute radiation exposure may affect colorectal carcinogenesis at very early stages, e.g., initial phases of the serrated polyp pathway which is closely related to the MSI phenotype.

To our knowledge only a single report on the molecular oncological analysis of radiation-associated colorectal cancer has been published to date.[34] This study involved the genetic analysis of five cases of radiotherapy-associated colorectal cancer and finding that the tumor specimens lacked MSI. It should be noted, however, that mucinous/poorly differentiated cancer specimens were not examined, and that the post-radiotherapy times were relatively short, i.e., between 12 and 26 years with median duration of 14 years. These times compared with the mean time since A-bombing (48.1 yrs) in our current study and median time since radiotherapy (27 yrs) for Japanese colorectal cancer cases with mucinous/poorly differentiated histology that had developed after pelvic irradiation.[4]

In considering these observations, we think that an extension of the follow-up period is essential for a better understanding of the pathological and molecular features of radiation-associated colorectal cancer including such cancer among A-bomb survivors.

Association between methylation of the *MLH1* gene and radiation dose was observed in this study (Figure 4). All five cases with the MSI-H phenotype showed LOH at the *MLH1* gene (Table 4). In addition, gene alterations relative to Ras-signaling also showed association with radiation dose (Figure 7). These results indicate a possible association between radiation exposure and gene alterations. Induction of delayed genetic alterations including somatic mutations, deletions, and chromosome aberrations by ionizing radiation has been demonstrated by several *in vitro* studies[35]: radiation-induced, late-arising mutations have been detected in descendant cells of established cell lines with long passage histories, mammary epithelium, and hematopoietic cells.[36,37,38] To date, several possible factors/processes, e.g., secretory factors, gap junctions, reactive oxygen species (ROS), etc., have been suggested by *in vitro* studies to mediate cell signals responsible for heritable changes after radiation exposure.[39,40,41] In addition, it has been reported that frequency of *AML1* gene mutations in myelodysplastic syndrome patients among A-bomb survivors diagnosed in 1995–2001 was significantly higher than that in the general population not exposed to radiotherapy.[42] These results and ours suggest that radiation may induce genetic alterations that play some role in the development of cancers, including colorectal cancers, among A-bomb survivors.

Effects of radiation on induction of global hypomethylation have been shown by both *in vitro* and *in vivo* experiments.[43,44] On the other hand, recent studies demonstrated that irradiation of cultured cells and mice can also induce hypermetylation of certain genes.[45,46] These observations of opposite epigenetic alterations by irradiation are similar to those of simultaneous global hypermethylation and gene specific hypermethylation in cancerous tissue.[47] Interestingly, the prevalence of *p16* gene methylation significantly increased as a function of plutonium dose in lung adenocarcinomas of plutonium-exposed workers in Mayak.[48] In the future, comparable tests of dose-dependency should be conducted using the molecular endpoints reported here for cases of A-bomb associated colorectal cancers among A-bomb survivors, but only when sufficient numbers of samples become available.

What factor(s) explain the preferential development of colon cancer among A-bomb survivors? Recent prospective nested case-control studies revealed an association between chronic low-grade inflammation (measured by the inflammatory marker C-reactive protein [CRP]) and development of colorectal cancer, i.e., colon cancer more than rectal

cancer.[49,50] Chronic low-grade inflammation may be one of the factors responsible for this noted preferential development of colon cancer over rectal cancer. A strong correlation between CRP levels and radiation dose has been reported for Adult Health Study (AHS) A-bomb survivor participants, with CRP levels increasing by about 31% per Gy of estimated A-bomb radiation.[51] Chronic inflammation provides sustained production of ROS.[52] Recently, a mechanistic link between inflammation and alterations of DNA methylation status has been proposed: 5-chlorocytosine, which is synthesized by chlorination of a cytosine residue by hypochlorous acid (HClO) released from inflammatory cells, mimics 5-methylcytosine to transfer a methyl-group to a cytosine residue within the opposite complementary strand CpG sequence by DNMT1.[53] Considering these results along with our own, we speculate that chronic low-grade inflammation caused by A-bomb radiation may be one factor affecting the preferential development of colon cancer over rectal cancer among A-bomb survivors.

Figure 8 summarizes our hypothesis of development of radiation-associated colorectal cancer among A-bomb survivors. Radiation exposure may directly induce ROS in the irradiated cells or indirectly via chronic inflammation, resulting in genomic instability and accumulation of mutations and deletions of genes associated with colorectal carcinogenesis, especially those related to the serrated polyp pathway. Radiation-induced inflammation may also generate HClO, increasing methylation of the genes associated with the pathway. To generate invasive carcinoma, a long duration of several decades would be required. As a result, the proportion of mucinous/poorly differentiated carcinoma to well/moderately differentiated cancer may differ by time since A-bombing. To test the hypothesis, analysis of molecular alterations in precancerous lesions such as hyperplastic polyp and serrated adenoma among A-bomb survivors will be necessary. Such tissues may already be available in the autopsy samples collected until 1985 at RERF.

In summary, the results obtained thus far imply that radiation exposure may influence MSI status and its related epigenetic and genetic alterations in colorectal carcinogenesis among A-bomb survivors, although further analysis with an increased number of cases is essential.

(**Notes:** Supplementary Tables are available upon request.)

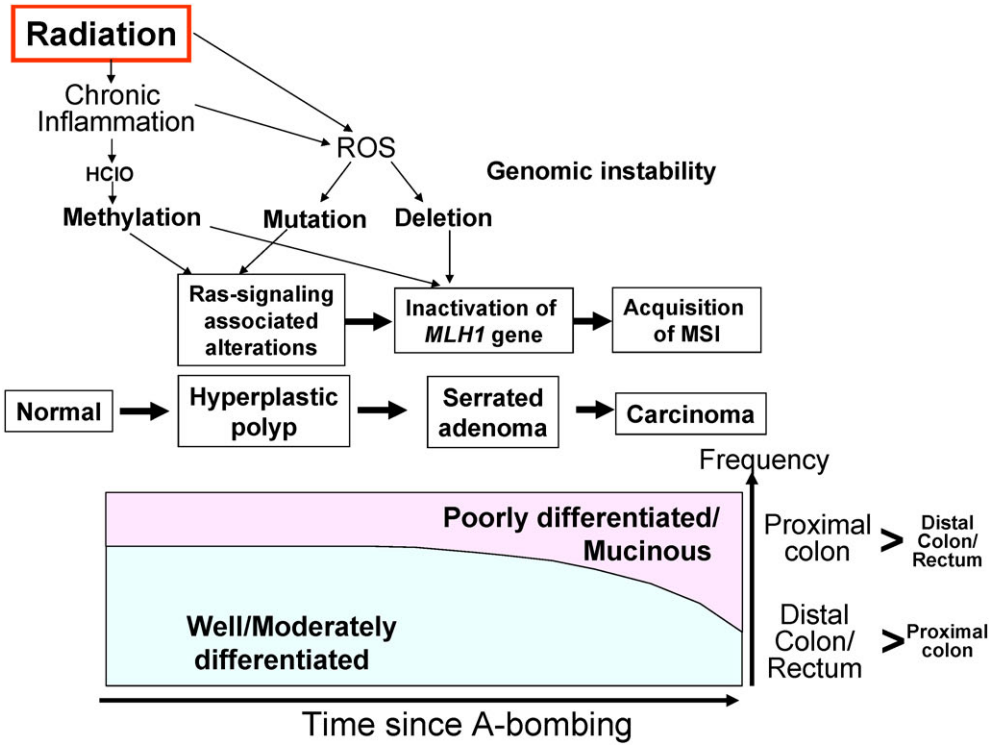

Figure 8. A hypothetical schema of the development of radiation-associated colorectal cancer among A-bomb survivors.

References

1. Thompson DE, Mabuchi K, Ron E, Soda M, Tokunaga M, Ochikubo S, Sugimoto S, Ikeda T, Terasaki M, Izumi S, Preston DL. Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958–1987. *Radiat Res* 137:S17-67, 1994.

2. Slaughter DP, Southwick HW. Mucosal carcinomas as a result of irradiation. *AMA Arch Surg* 74:420-9, 1957.

3. Castro EB, Rosen PP, Quan SH. Carcinoma of large intestine in patients irradiated for carcinoma of cervix and uterus. *Cancer* 31:45-52, 1973.

4. Narui K, Ike H, Fujii S, Nojiri K, Tatsumi K, Yamagishi S, Saito S, Kunisaki C, Imada T, Nozawa A, Ohki S, Ota M, Ichikawa Y, Shimada H. A case of radiation-induced rectal cancer. *Nippon Shokakibyo Gakkai Zasshi* 103:551-7, 2006. (Japanese)

5. Soreide K, Janssen EA, Soiland H, Korner H, Baak JP. Microsatellite instability in colorectal cancer. *Br J Surg* 93:395-406, 2006.

6. Kuismanen SA, Holmberg MT, Salovaara R, de la Chapelle A, Peltomaki P. Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. *Am J Pathol* 156:1773-9, 2000.

7. Deng G, Chen A, Hong J, Chae HS, Kim YS. Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. *Cancer Res* 59:2029-33, 1999.

8. Furukawa T, Konishi F, Masubuchi S, Shitoh K, Nagai H, Tsukamoto T. Densely methylated MLH1 promoter correlates with decreased mRNA expression in sporadic colorectal cancers. *Genes Chromosomes Cancer* 35:1-10, 2002.

9. Miyakura Y, Sugano K, Konishi F, Ichikawa A, Maekawa M, Shitoh K, Igarashi S, Kotake K, Koyama Y, Nagai H. Extensive methylation of hMLH1 promoter region predominates in proximal colon cancer with microsatellite instability. *Gastroenterology* 121:1300-9, 2001.

10. Hawkins NJ, Ward RL. Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. *J Natl Cancer Inst* 93:1307-13, 2001.

11. O'Brien MJ, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amorosino M, Farraye FA. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol* 30:1491-501, 2006.

12. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology* 50:113-30, 2007.

13. Vos MD, Ellis CA, Elam C, Ulku AS, Taylor BJ, Clark GJ. RASSF2 is a novel K-Ras-specific effector and potential tumor suppressor. *J Biol Chem* 278:28045-51, 2003.

14. Akino K, Toyota M, Suzuki H, Mita H, Sasaki Y, Ohe-Toyota M, Issa JP, Hinoda Y, Imai K, Tokino T. The Ras effector RASSF2 is a novel tumor-suppressor gene in human colorectal cancer. *Gastroenterology* 129:156-69, 2005.

15. Hesson LB, Wilson R, Morton D, Adams C, Walker M, Maher ER, Latif F. CpG island promoter hypermethylation of a novel Ras-effector gene RASSF2A is an early event in colon carcinogenesis and correlates inversely with K-ras mutations. *Oncogene* 24:3987-94, 2005.

16. Young RW, Kerr GD, eds. *Reassessment of the Atomic Bomb Radiation Dosimetry for Hiroshima and Nagasaki—Dosimetry System 2002*. Radiation Effects Research Foundation, Hiroshima, 2005.

17. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 58:5248-57, 1998.

18. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, Seruca R, Lacopetta B, Hamelin R. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. *Gastroenterology* 123:1804-11, 2002.

19. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 96:261-8,

2004.

20. Takahashi K, Eguchi H, Arihiro K, Ito R, Koyama K, Soda M, Cologne J, Hayashi Y, Nakata Y, Nakachi K, Hamatani K. The presence of BRAF point mutation in adult papillary thyroid carcinomas from atomic bomb survivors correlates with radiation dose. *Mol Carcinog* 46:242-8, 2007.

21. Xiong Z, Laird PW. COBRA: a sensitive and quantitative DNA methylation assay. *Nucleic Acids Res* 25:2532-4, 1997.

22. Li LC, Dahiya R. MethPrimer: designing primers for methylation PCRs. *Bioinformatics* 18:1427-31, 2002.

23. Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res* 29(17):E88-8, 2001.

24. R Development Core Team (2005). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

25. Raut CP, Pawlik TM, Rodriguez-Bigas MA. Clinicopathologic features in colorectal cancer patients with microsatellite instability. *Mutat Res* 568:275-382, 2004.

26. Nagasaka T, Sasamoto H, Notohara K, Cullings HM, Takeda M, Kimura K, Kambara T, MacPhee DG, Young J, Leggett BA, Jass JR, Tanaka N, Matsubara N. Colorectal cancer with mutation in BRAF, KRAS, and wild-type with respect to both oncogenes showing different patterns of DNA methylation. *J Clin Oncol* 22:4584-94, 2004.

27. Fransen K, Klintenas M, Osterstrom A, Dimberg J, Monstein HJ, Soderkvist P. Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas. *Carcinogenesis* 25:527-33, 2004.

28. Tanaka H, Deng G, Matsuzaki K, Kakar S, Kim GE, Miura S, Sleisenger MH, Kim YS. BRAF mutation, CpG island methylator phenotype and microsatellite instability occur more frequently and concordantly in mucinous than non-mucinous colorectal cancer. *Int J Cancer* 118:2765-71, 2006.

29. Koinuma K, Shitoh K, Miyakura Y, Furukawa T, Yamashita Y, Ota J, Ohki R, Choi YL, Wada T, Konishi F, Nagai H, Mano H. Mutations of BRAF are associated with extensive hMLH1 promoter methylation in sporadic colorectal carcinomas. *Int J Cancer* 108:237-42, 2004.

30. Li WQ, Kawakami K, Ruszkiewicz A, Bennett G, Moore J, Lacopetta B. BRAF mutations are associated with distinctive clinical, pathological and molecular features of colorectal cancer independently of microsatellite instability status. *Mol Cancer* 5:2, 2006.

31. Arai T, Takubo K, Sawabe M, Esaki Y. Pathologic characteristics of colorectal cancer in the elderly: a retrospective study of 947 surgical cases. *J Clin Gastroenterol* 31:67-72, 2000.

32. Nakatsuka H, Shimizu Y, Yamamoto T, Sekine I, Ezaki H, Tahara E, Takahashi M, Shimoyama T, Mochinaga N, Tomita M, Tsuchiya R, Land CE. Colorectal cancer incidence among atomic bomb survivors, 1950–80. *Radiat Res (Tokyo)* 33:42-61, 1992.

33. Rotmensch S, Avigad I, Soffer EE, Horowitz, A, Bar-Meir S, Confino R, Czerniak A, Wolfstein I. Carcinoma of the large bowel after a single massive dose of radiation in healthy teenagers. *Cancer* 57:728-31, 1986.

34. Tsuji T, Sawai T, Nakagoe T, Hidaka S, Shibasaki S, Tanaka K, Nanashima A, Yamaguchi H, Yasutake T, Tagawa Y. Genetic analysis of radiation-associated rectal cancer. *J Gastroenterol* 38:1185-8, 2003.

35. Wright EG, Coates PJ. Untargeted effects of ionizing radiation: implications for radiation pathology. *Mutat Res* 597:119-32, 2006.

36. Little JB, Nagasawa H, Pfenning T, Vetrovs H. Radiation-induced genomic instability: delayed mutagenic and cytogenetic effects of X rays and alpha particles. *Radiat Res* 148:299-307, 1997.

37. Selvanayagam CS, Davis CM, Cornforth MN, Ullrich RL. Latent expression of p53 mutations and radiation-induced mammary cancer. *Cancer Res* 55:3310-7, 1995.

38. Harper K, Lorimore SA, Wright EG. Delayed appearance of radiation-induced mutations at the Hprt locus in murine hemopoietic cells. *Exp Hematol* 25:263-9, 1997.

39. Nagar S, Smith LE, Morgan WF. Characterization of a novel epigenetic effect of ionizing radiation: the death-inducing effect. *Cancer Res* 63:324-8, 2003.

40. Nagar S, Morgan WF. The death-inducing effect and genomic instability. *Radiat Res* 163:316-23, 2005.

41. Persaud R, Zhou H, Baker SE, Hei TK, Hall EJ. Assessment of low linear energy transfer radiation-induced

bystander mutagenesis in a three-dimensional culture model. *Cancer Res* 65:9876-82, 2005.

42. Harada H, Harada Y, Tanaka H, Kimura A, Inaba T. Implications of somatic mutations in the AML1 gene in radiation-associated and therapy-related myelodysplastic syndrome/acute myeloid leukemia. *Blood* 101:673-80, 2003.

43. Kalinich JF, Catravas GN, Snyder SL. The effect of gamma radiation on DNA methylation. *Radiat Res* 117:185-97, 1989.

44. Pogribny I, Raiche J, Slovack M, Kovalchuk O. Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. *Biochem Biophys Res Commun* 320:1253-61, 2004.

45. Kovalchuk O, Burke P, Besplug J, Slovack M, Filkowski J, Pogribny I. Methylation changes in muscle and liver tissues of male and female mice exposed to acute and chronic low-dose X-ray-irradiation. *Mutat Res* 548:75-84, 2004.

46. Kaup S, Grandjean V, Mukherjee R, Kapoor A, Keyes E, Seymour CB, Mothersill CE, Schofield PN. Radiation-induced genomic instability is associated with DNA methylation changes in cultured human keratinocytes. *Mutat Res* 597:87-97, 2006.

47. Ehrlich M. DNA methylation in cancer: too much, but also too little. *Oncogene* 21:5400-13, 2002.

48. Belinsky SA, Klinge DM, Liechty KC, March TH, Kang T, Gilliland FD, Sotnic N, Adamova G, Rusinova G, Telnov V. Plutonium targets the p16 gene for inactivation by promoter hypermethylation in human lung adenocarcinoma. *Carcinogenesis* 25:1063-7, 2004.

49. Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. *JAMA* 291:585-90, 2004.

50. Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S; Japan Public Health Center-Based Prospective Study Group. Plasma C-reactive protein and risk of colorectal cancer in a nested case-control study: Japan Public Health Center-based prospective study. *Cancer Epidemiol Biomarkers Prev* 15:690-5, 2006.

51. Hayashi T, Kusunoki Y, Hakoda M, Morishita Y, Kubo Y, Maki M, Kasagi F, Kodama K, Macphee DG, Kyoizumi S. Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors. *Int J Radiat Biol* 79:129-36, 2003.

52. Pavlick KP, Laroux FS, Fuseler J, Wolf RE, Gray L, Hoffman J, Grisham MB. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. *Free Radic Biol Med* 33:311-22, 2002.

53. Valinluck V, Sowers LC. Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. *Cancer Res* 67:946-50, 2007.

The "Old Boys' Club"—Report on a Series of Interviews with Former ABCC Employees

Well, they jokingly refer to themselves as belonging to the "Old Boys' Club," but their good natured demeanor, quick minds, and lively steps all belie this label—belie this membership. However, we're getting ahead of ourselves in relaying to you how this article came about. Sometime ago, Ikawa-san and I wanted to provide the readers of the *Update* with a special treat—a "human interest" story on what it was like during the early days of ABCC and decided that an interview (or series of interviews) with several prominent former ABCC employees might just do the trick. So with this in mind we set upon the task (the pleasure) of talking to five delightful gentlemen.

On May 17th, we met with three former employees from the Hiroshima Laboratory—Mr. Michael E. Rappaport, a former senior administrator, Mr. Isao Moriyama, a former chief of the General Affairs Section, and Mr. Shizuo Inoue, a former assistant chief of the Secretariat. Approximately a month later, on June 22nd, following the annual RERF Board of Directors meeting in Nagasaki, I along with Dr. Evan Douple of the U.S. National Academy of Sciences (NAS), had a chance to interview two former ABCC employees from the Nagasaki Laboratory, namely Mr. Masayuki Hayashida, former operations administrator, and Mr. Yoshio Okamoto, a General Affairs Section chief and later the operations administrator.

For both interviews we asked the same basic set of six questions relating to: a) how they came to work at ABCC, b) the lab's organization, operations, and contact with notable personnel, c) major operational problems, including the issue of bi-national nature of the lab and relationships between Hiroshima and Nagasaki labs, d) difficulties in contacting and enlisting cooperation of A-bomb survivors, e) treatment and care of study participants, f) thoughts on RERF's present state and future directions.

[Editors note: The responses listed and attributed to the former employees are not all direct quotes, but often represent paraphrased and interpreted responses taken from notes collected during the interviews. We trust that we've reported accurately the intent of the employees' comments, if not their exact words.]

Questions to the "Old Boys" and Their Comments

How you happened to come to work for ABCC?

Mr. Rappaport recalled that in 1947 he was a staff officer in the office of chief engineer of the British Commonwealth Occupation Force on Etajima. The office was responsible for occupation force construction in Chugoku and Shikoku region as well as for the Supreme Commander for the Allied Powers Military Government and other organizations such as ABCC. Later his office moved to Kure and there he got to know Dr. Carl Tessmer, ABCC's first director who periodically visited his office to check on the progress of work on the temporary laboratory at Ujina and maintenance of rented staff housing. About that time Australian Forces were drawing down and it was time to think of returning to Australia. He had no enthusiasm for returning to his old job in a copper mine and then Dr. Tessmer offered him a job for one year as an associate engineer on the construction of the original Hijiyama Laboratory. He accepted it without hesitation.

His contract was extended for two more years during which he was in charge of construction of additional buildings (Buildings G and H) in Hijiyama using materials sent to Japan for construction of Kure clinic. Early in 1949, NAS created the position of "Business Administrator" responsible for budget and personnel reporting to the ABCC director and the NAS business manager. He was appointed to that position and continued it until transition from ABCC to RERF in 1975. Upon his retirement (1986), Mr. Richard Sperry succeeded him as business administrator.

Moriyama-san told us that he started his work at ABCC in January 1948. Following his Navy duty, he searched for gainful employment and a neighbor friend (in Kure) suggested ABCC as a possibility. The neighbor happened to work at Health Department of Kure City Office, and assisted ABCC in its survey of control subjects. Moriyama-san said that ABCC attracted him because "…it was work and it paid well…." He initially applied for a position as a medical technician; but once employed, he worked as an ABCC "contactor" of survivors for possible enrollment as study subjects. He worked six days per week: Monday, Wednesday, and Friday he worked in Hiroshima trying to enroll A-bomb-exposed survivors; while on Tuesday, Thursday and

Saturday he worked to enroll non-exposed individuals in Kure. Wednesday and Saturday were apparently half days. During this period, ABCC was located in the two rooms on the 2nd floor of the Red Cross Hospital in Hiroshima, and at the Kyosai Hospital in Kure. The unit had about 30 staff in total, including nurses, clerks, and contactors. Dr. Koji Takeshima, who had a concurrent assignment between the Red Cross Hospital and ABCC, was responsible for the work of the contactors and their visits to survivors with epilation. Moriyama-san said that he visited about 10 or 15 persons a day both in Hiroshima and Kure, and of the survivors contacted, only about half were willing to participate.

Inoue-san indicated that he started work at ABCC in 1949. Just prior to coming to ABCC, he was employed by a telephone company on Miyajima. The opportunity to work at a research facility and to learn new things was the major factor that attracted him to ABCC; although, he added that simply having a good job was undeniably important as well. He was interviewed by Mr. Hamako, a Japanese-American. He worked initially as a dispatcher in the "motor pool" which had approximately 100 people, including mechanics and drivers (ABCC Ujina lab). To put this number into proper perspective, in the 1950 time frame ABCC had approximately 1,000 employees, with 700 staff in Hiroshima and 300 staff in Nagasaki.

Hayashida-san joined ABCC Nagasaki as a clerk in 1950 at the tender age of 25 years. He indicated that his primary interest in working for ABCC was the opportunity to learn English, and at the time the lab with its staff of about 150 had a fair number of Americans. Just after the war, upon returning home, he had worked part time at a laundry reception counter of the Allied Occupation Forces office in the city. Later he was employed at a local fish company (currently affiliated with the large trading company Mitsui & Co.) doing clerical work, but the company closed and he was forced to find other employment.

(From left) Mr. Shizuo Inoue, Mr. Isao Moriyama, Ms. Yuko Ikawa (editor), and Mr. Michael E. Rappaport at the Hiroshima Laboratory

Due to his mother's illness, he sought work in the Nagasaki area [this comment prompted us to ask, whether his mother's illness was due to the A-bomb]. He said that his mother was not exposed, due to the fact that toward the end of the war, women and children were evacuated to Shimabara. Hayashida-san also went on to say that he was not an A-bomb survivor, but was away from Nagasaki at the time of the bombing serving in the military.

Okamoto-san joined ABCC Nagasaki in 1956 as a "field investigator" and worked in this capacity for about a year. He explained that in the 1950 National Census an interview survey was conducted for A-bomb survivors, and it was from this survey that A-bomb survivors were identified for possible inclusion in the ABCC study population. He and the other field investigators (a team of ten) had the responsibility of locating, visiting, and interviewing people who were identified as A-bomb survivors. Using forms of the master sample questionnaire, the team collected information regarding family composition and exposure status, as well as medical information regarding symptoms after exposure to the bomb. About a year later he was transferred to another section that was engaged in shielding surveys. Specially designed questionnaires were used to document exposure status of the survivors. The team focused on those survivors (about 12,000 in total) who were exposed fairly close (within 2,000 m) to the hypocenter. Only about 80% of these individuals could be interviewed; about 20% were unavailable due to a variety of reasons (i.e., some simply declined, others had passed away, or moved outside Nagasaki City.) These interviews revealed that about one-third of these individuals were inside their own homes at the time of the bombing, while the remaining people were exposed outside, or inside concrete buildings or other structures. *We asked* "Was there a problem—and issue—concerning the survivor's uncertainty-of-location and their 'recall' ?" "Yes…" he replied, but only occasionally. The problem was solved largely by multiple visits and conducting additional interviews.

We were curious about whether some individuals were sheltered, or in actual bomb shelters. Again he answered "…yes…, some individuals were indeed sheltered, and were spared, despite being fairly close to the hypocenter."

Because of his work and interest in shielding and dosimetry, he was sent to the Oak Ridge National Laboratory (Tennessee, U.S.) for additional education and training. It was during this time frame (circa 1957) that the so-called dosimetry-based Ichiban Project was in progress, and as a result Okamoto-san had a chance to interact with a small number of U.S. radiation scientists who took turns visiting Hiroshima and Nagasaki for well over a year and half and provided guidance to ABCC on shielding

(From left) Dr. Thomas Seed (editor), Mr. Masayuki Hayashida, Dr. Evan Douple of NAS, and Mr. Yoshio Okamoto at the Nagasaki Laboratory

(e.g., by concrete construction materials) and dosimetry of A-bomb radiation.

Finally *we asked* whether he had seen the new DS02 report. He indicated that he had seen it.

Can you tell us little about ABCC's organization, operations, and your contact with notable personnel?

Mr. Rapapport (on the issue of satellite labs) commented that four labs, of identical design, were originally planned: Hiroshima and Kure, with the Kure lab serving as the "control lab" for the unexposed cohort; and Nagasaki and Sasebo, with the latter serving to monitor the control, unexposed cohort. At first there were plans to include subjects living in the four prefectures of each of the Chugoku and Shikoku districts, but also in Tokyo and Yokohama as well. There was a plan to build a lab in Uragami, near the hypocenter in Nagasaki. An initial survey of the area was made, but the project never got past the design stage just as Kure and Sasebo.

Moriyama-san indicated that the plans for the Kure lab were cancelled due to budget problems, as was the Sasebo lab in the Nagasaki area.

Inoue-san followed up by saying that in fact, operational elements of ABCC did exist in Kure, e.g., a motor pool with dispatchers. Inoue-san also said (on the issue of the Hijiyama site) that despite a common misimpression, the ABCC site was indeed used militarily by the Japanese, and had full intention of installing anti-aircraft guns on the site. However, the site was prepared but the guns never installed.

Mr. Rapapport indicated that York and Sawyer (a New York City firm) was original design firm for the Hijiyama lab. When asked about the interesting "quonset hut" design, and whether it was the only design considered, he responded that "…at the time, the thought was that this design was the most appropriate for rapid installation." Further, he said that the plans to obtain the construction materials by the U.S. army in Okinawa fell through, and in the end, the construction materials were all secured and transported from the U.S. Although he was not sure of the exact cost of the facilities, he estimated it at somewhere between $500,000 and $1,000,000. Takenaka Komuten Co. was awarded the building contract, and when the work was completed, the company ended up winning the "Contractor of the Year" (a Japanese award). Mr. Rapapport went on to say that Hijiyama Hall (the residence hall) by contrast, was designed by a Japanese architect, Kunio Maekawa, and constructed by Shimizu Kensetsu Co.

Inoue-san commented on the salaries and budget: the annual budget, circa 1950, was approximately $100,000. By 1975 the budget had risen to $8 million, and by 2006 it topped $38 million. **Moriyama-san** added the comment that the average salary in Japan during the 1950 time frame was 700–800 yen per month, and that ABCC was offering approximately 1,500 yen per month, in addition to the offer of a possible 50 hours of paid overtime (that equated to an additional 1,000 yen extra per month). As consequence, staff recruitment at ABCC during these early years was not a problem. The willingness of people to stand on long lines for extended periods when jobs were offered, attested to the latter. However, all three gentlemen indicated that at the time, ABCC employment was considered fairly tenuous (with the labor union being organized somewhat later in 1953): all three chuckled at this thought, since these gentlemen (and many others) found life-times of very stable employment.

ABCC covered the salary difference between ABCC and the National Institute of Health (NIH) employees (ABCC: 1,500 yen, NIH: 1,000 yen in 1947), until the reorganization in 1975. NIH's involvement was deemed essential for ABCC research operations (in particular for the Life Span Study and access to "koseki"), as NIH served as a vital liaison function for the organization in dealing with the Ministry of Health and Welfare (MHW) and local governments.

Hayashida-san (on the issue of ABCC facilities in Nagasaki) commented that by the time he was hired by ABCC (in November of 1950), ABCC Nagasaki had already (Spring of 1949) relocated to the Nagasaki Prefectural Kyoiku Kaikan. Prior to this, ABCC was located inside the Shinkozen Primary School, which had been made into a hospital during the war and from which ABCC initiated its activities. "What about the capacity of the Prefectural Kyoiku Kaikan? Do you remember how many study subjects visited ABCC per day?" *we asked*. Hayashida-san stated that when ABCC was initially established, approximately 25 to 30 persons visited it per day, although the number of study participants varied considerably. There was a capacity to handle

even more participants, but the number of participants seen each day was intentionally limited so that more time could be spent for each individual and that each individual could be treated more respectfully. "I believe that in those days, we accepted, on average, 20 subjects or so per day." "Despite our efforts, some people complained that ABCC was forceful in bringing study subjects to the institution. Therefore, with such a complaint in mind, I think we were determined not to specify the time for anyone to visit ABCC for examinations. We asked our study subjects when they would be available and told them that an automobile would be sent to them for transportation to and from ABCC when it was convenient for them."

Mr. Rapapport (on the issue of notable persons) indicated (as did Moriyama-san and Inoue-san) that he had no first-hand knowledge of the activities of initial research group that surveyed the bombed cities and worked out of the so-called "disaster train" in 1946 under the auspices of the U.S. National Research Council. [Editor's note: This group included two civilian radiobiologists, Drs. Paul Henshaw and Austin Brues, three U.S. medical officers, Drs. James Neel, Melvin Block, and Frederick Ullrich. There were several Japanese researchers/physicians as well, most notably Dr. Masao Tsuzuki, a prominent professor of surgery at Tokyo University.] Nevertheless, Mr. Rapapport provided an interesting comment on several notables during the subsequent, early period of ABCC. He related a story he had been told early on about the invaluable work of Dr. Tsuzuki in persuading local doctors in both Hiroshima and Nagasaki to assist ABCC in their attempts to conduct medical follow-up studies of the A-bomb survivors. Quite understandably, many of the local physicians were either hesitant, or outright refused, to assist in the effort, and it was only through Dr. Tsuzuki's considerable influence was this problem resolved. Apparently, the famous doctor, Dr. Takashi Nagai of Nagasaki University, was just one of many doctors that Dr. Tsuzuki talked into helping ABCC out. Mr. Rappaport pointed out the contrasting personalities and interests of ABCC's first director, Dr. Tessmer and Dr. Neel, the prominent researcher who was largely responsible for establishing the clinical research agenda at the new lab. "The differences were pronounced: Dr. Tessmer was truly dedicated in terms of trying to set up and to manage the new lab (ABCC), whereas Dr. Neel seemed more interested in the research, and less in management." He also commented that Dr. Henshaw was the person who named the laboratory, i.e., the Atomic Bomb Casualty Commission (ABCC), and served as a liaison between NAS and ABCC. However, except for relatively short visits, his stay in Hiroshima was limited.

Hayashida-san commented on Dr. Raisuke Shirabe, former professor of Nagasaki University, an ABCC personality of note. "…I knew Dr. Shirabe very well. We were both heavy smokers, and during teatime starting at 3 o'clock, he would often ask me to share a smoke with him. During these breaks, I learned a lot about him and his various activities…" "…having a meticulous nature, Dr. Shirabe made entries in his diary every day." "…I believe that Dr. Shirabe understood ABCC studies in Nagasaki better than anyone, and was remarkably cooperative in the conduct of the ABCC-related studies." "Dr. Shirabe played a key role in ABCC's early years, especially in terms of recruiting key physicians and other Nagasaki University graduates for ABCC. This was particularly remarkable in light of the fact that his two sons fell victim to the atomic bombing while they were at the Nagasaki University Faculty of Medicine, and he himself was an A-bomb survivor." Hayashida-san commented that Dr. William J. Schull considered himself a disciple of Dr. Shirabe. Apparently, Dr. Schull in his first meeting with Dr. Shirabe (at the time ABCC Nagasaki was working out of the Shinkozen Primary School), he was deeply impressed by the man and by "…finding such an excellent physician in Japan." Hayashida-san further commented that Dr. Schull's modesty really impressed him, as did the remarkable nature of Dr. Shirabe. In passing, Hayashida-san mentioned that he thought Dr. George Darling (ABCC's fifth director) to be "…quite remarkable as well." However, Hayashida-san seemed to suggest that it was Dr. Shirabe whom he most admired: "I read Dr. Shirabe's diary in an attempt to understand how it was at the time of the bombing. During the complete confusion following the bombing, while treating the injured, Dr. Shirabe calmly took notes about the damage and the weather, including wind direction and temperature. I had the impression that Dr. Shirabe was a true scientist."

Okamoto-san told a different story about Dr. Shirabe; one he considered less scientific, and somewhat amusing (at least at this point in time). The story goes that Dr. Susumu Tsunoo, the then president of Nagasaki University, was severely injured due to the bombing and headed north to find shelter. Being a good friend of Dr. Tsunoo, Dr. Shirabe accompanied him for medical treatment, but despite treatment, several days later, Dr. Tsunoo died. Dr. Shirabe feeling sick himself (physically sick and mentally anguished about loss of his good friend) consoled (and self-medicated) himself by drinking a lot of *shochu* (distilled alcohol) in his bidding a good and final farewell to this world. But surprisingly, he found himself feeling better in subsequent days and eventually fully recovered. "In relaying this story to me, he told me (quite jokingly) that copious amounts of alcohol might be an effective medicinal for radiation sickness."

Would you care to comment on the bi-national nature of ABCC? Were there personnel issues? …communication problems? …operational problems? …How was the relationship between the two ABCC facilities, Hiroshima and Nagasaki?

Inoue-san (on the bi-national issue) "In my personal opinion, there was no problem at all." Inoue-san indicated that much like today, the Japanese staff worked very well together with the small contingent of U.S. staff.

Moriyama-san commented on the fact that the Japanese staff lived in locations different from that of the U.S. staff, perhaps contributing to a sense of a "cultural divide" between the U.S. and Japanese staff. The U.S. contingent in Kure, for example, lived in an area meant for personnel associated with the occupation, i.e., the "Niji-mura." Interestingly, ABCC provided free bus service for staff living in these outer areas.

Mr. Rapapport added to Moriyama-san's comment by telling us that he was one of those staff members who lived in a rented house in Kure and used the bus service that Moriyama-san mentioned. He also said that there was some minimal resentment over differences in the level of positions held by U.S. and Japanese staff. Many of Japanese-Americans were bilingual and were placed in supervisory positions.

Hayashida-san and **Okamoto-san** both said that in Nagasaki at least, the Japanese and the U.S. employees got along very well, and there were amazingly few problems. They reminisced about the Christmas parties in which a small group of resident Americans would invite the Nagasaki staff to join in their celebrations. Although there was an "oddball" or two along the way, for the most part, the U.S. staff fit in quite nicely, as they were generally kind, had families, and played sports (baseball and swimming) with the Japanese staff.

Hayashida-san remembers that Dr. Darling was a good leader, and tried his best to get people working together during these early days of ABCC. However, when MHW became actively engaged, the organization became much more bureaucratic. ABCC Hiroshima was "…very prescriptive" in its approach to management, and would dictate very detailed, specific instructions for various programmatic activities.

Moriyama-san (on the issue of Hiroshima-Nagasaki relationship) indicated that working relationships and communications between the two labs were quite good, despite the distance, often with less than ideal communication systems (compared to today's systems), and with differences in daily operations. For example, pay day was different in Hiroshima (on the 30th of each month) than it was in Nagasaki (on the 15th), and since personnel management and salary calculations for both labs were made in Hiroshima, Moriyama-san had to travel to Nagasaki twice every month in order to make these payrolls, and to conduct a variety of other business transactions. Each trip required well over a day (a total 24 hours for each roundtrip).

What were the difficulties in contacting and enlisting the cooperation of the A-bomb survivors themselves?

Moriyama-san talked about his very early experience while at the Red Cross Hospital and the Ujina lab in the contacting effort. He visited 10–15 persons a day, and scheduled appointments for clinical exams a week in advance for these individuals. However, only about a half of them agreed to come to ABCC. Probably due to the difference in disaster of A-bombing, persons exposed more distally (living in the suburbs) tended to be more cooperative.

Inoue-san said that he was not directly involved in the "contacting work"; however, as a result of his "motor pool" work, he was told by the drivers about the survivor's pleasure of being picked up and sent back home by "fancy limousine," and by being treated very nicely by ABCC staff. He indicated that the vast majority of the survivors were very cooperative and willing to participate, and this helped enormously in gathering "subjects" for both ongoing and future studies. Despite this, some survivors unquestionably felt some pressure to participate due to the authority and direction of the "occupying" U.S. military. However, because of the well-recognized utility of the ABCC studies, feeling good about participating was less of a problem for most of the survivors. Another drawing point was that many of the survivors were anxious about their health, and that ABCC would provide free medical exams and consultations. However, this was countered by their knowing that only exams, and no treatments, were to be given. As a result of the latter, some people harbored bad feelings about ABCC's operations.

Okamoto-san commented on the problems in contacting and handling of "survivors" at Nagasaki clinical facilities. *We asked* "Was it difficult to persuade 'survivors' to participate?" "Yes, at times…," and "…although the number was small, some people simply declined to be interviewed, apparently because they did not want to say anything at all about their experiences." Okamoto-san indicated that the "contactors" were successful, more often than not, in convincing survivors to participate in ABCC's medical program, but many times this would require visiting individuals several times and trying to communicate the worthiness of ABCC's medical program. "At times, I would use my wife as an example, saying that she is also an A-bomb survivor and is enrolled in the program, and believes that the program is highly beneficial not only to the survivors themselves, but to their children. By quiet, gentle,

but full explanation of the program's virtues, we were generally successful in getting survivors to understand ABCC's intent and to have them participate in the program."

It sounds to us as if you were acting as a "goodwill ambassador" for the program. "Yes, I believe so…" Subsequently, **Okamoto-san** told this story about the same newspaper reporter who would visit the foundation almost every year, and would incessantly ask "Isn't RERF taking advantage of A-bomb survivors by conducting only research?" Okamoto-san would explain to the reporter that RERF is not like that at all—"Some of the RERF employees are A-bomb survivors, though the number is small, and more than half of the employees have family members who are A-bomb survivors. We all believe in the positive nature of the program, otherwise we would not be so actively engaged and encouraging our family members to participate." Okamoto-san followed this comment up by saying "…prejudice of this kind has been reduced significantly, largely by enormous effort made by contactors since the ABCC days to maintain close relationships with study subjects."

The treatment issue referred to previously still lingers—Can you shed any light on this issue? Were there, or were not, treatments given to the participants?

Moriyama-san, Mr. Rappaport, and **Inoue-san** all indicated that at certain periods, under very limited situations, treatments were provided. They suggested that limited treatments were provided when needed, and referred to the beds in clinic area and the small number of patients (about 15) that received treatments around 1963–1965 when Dr. Darling was director, but this program did not continue long (e.g., Mr. Rapapport cited drugs for leukemia being given at times because ABCC had access to the newer medicines coming from the U.S. that were not readily available in the Hiroshima/Nagasaki regions.

We asked about the thoroughness of the ABCC doctors in following up on the health status of participants that were patently ill. Inoue-san cited the stories he had heard from the survivors themselves, about ABCC doctors recommending further consultations with medical specialists at local hospitals when warranted. He also mentioned being told by ABCC drivers just how appreciative a number of survivors of ABCC in the providing checkups that lead to diagnoses and successful treatments of given life-threatening diseases.

Moriyama-san confirmed Inoue-san's comments that some of the survivors complained that ABCC did not provide any treatment. However, he added that although the services ABCC provided were limited, they were exceedingly important and useful to the survivors (in terms of clinical exams and diagnoses). Importantly, ABCC gave the survivors much needed instruction and guidance on seeking and securing proper help on their medical problems. As a result, the majority of the survivors appreciated this service and the kindness of ABCC staff.

Mr. Rappaport commented that he thought that relationships between the doctors and the study subjects were good, and that the nurses were very kind to the subjects. He followed this up by saying that at one point, ABCC actually employed social workers to help the "survivors" handle personal problems that they were facing in daily lives. Considering the rarity of "social workers" in Japan during this period, it points to ABCC being a caring and well-meaning institution.

Hayashida-san (on the treatment question) said that in Nagaskai, A-bomb survivors were contacted and were brought by automobile to ABCC. There were about three station wagons and 25 jeeps at ABCC. "Our contactors visited the homes of A-bomb survivors and experienced a range of problems, but always tried to handle the interview and recruiting of the survivors sensitively. The same was true at ABCC itself, in that the staff treated the survivors kindly and politely, in a manner totally different from the way patients are treated in hospitals in Japan." Hayashida-san went on to say "I do not know whether the word 'patient' is even correct or not: I once proposed use of the word 'participants' in order to avoid any misunderstanding (of what ABCC was about). I was instructed by the Personnel Section in Hiroshima by phone to employ the most friendly and competent persons as receptionists. ABCC regarded the Reception (of the participants) as an important section. I never heard complaints from A-bomb survivors who visited ABCC about their treatment, although they seemed to have some feelings about the damage they suffered from the atomic bombing."

In following up on Hayashida-san's comments, *we asked* whether or not ABCC Nagasaki had facilities for accommodating persons who were found to have health problems during the treatment and recovery phases of their illnesses? **Hayashida-san** answered that "…there were no such treatment facilities at ABCC Nagasaki. However, at ABCC Hiroshima, there were such facilities, but only for a relatively short period of time. ABCC was not an institution that ill people visited. If any disease was found, we referred the patients to a physician and provided them with their own health examination data."

Dr. Douple specifically commented that "Around the time when ABCC was established, U.S. and Japanese government officials thought that it would be unwise for U.S. involvement to disturb the Japanese medical system, within which Japanese physicians had established the style of and rights for conducting treatment and achieved credibility among

their patients. It was therefore agreed between the two parties that ABCC would not conduct treatment and that, if any health problems were found, patients would be referred to specific hospitals. A long time has passed since such decisions were made. Do you believe that those decisions were appropriate?" **Hayashida-san** mentioned "I've heard of such decisions. I frequently heard complaints from not only A-bomb survivors, but also other citizens to the effect that ABCC conducted examinations but provided no treatment. …However, I think that the relationship between the local medical community in Nagasaki and ABCC was very good."

We asked about the often-leveled criticism by some A-bomb survivors that ABCC was an institution that treated them as guinea pigs. "When study participants visited ABCC for their health examinations, and they were diagnosed as having a given disease, were they instructed to visit hospitals? Did ABCC confirm that those study participants actually visited hospitals?"

Hayashida-san "ABCC physicians wrote referrals for such individuals: they could not specify hospitals. Therefore, the physicians generally suggested to those individuals that they go to the University Hospital, or to hospitals near their residence for additional examinations, or alternatively offered to write referrals to the survivor's hospital of choice." Participants were given their ABCC-generated clinical records (e.g., X-ray films etc.) so that they could provide these records, for review purposes, to the physician(s) of the referred hospital or clinic. Hayashida-san also commented that temporary medicines were provided in order to help them through the transition period from the ABCC diagnosis to seeing their primary physician at the local hospital or clinic. Okamoto-san added "…by providing guidance to A-bomb survivors, physicians at ABCC improved the institution's credibility among the survivors."

We asked "Why do you think some of the A-bomb survivors continue to think that ABCC-RERF has treated them as guinea pigs?"

Okamoto-san "I do not think that there is still such a sentiment among this group. I believe that there is a high level of understanding and confidence placed in RERF. If some people think that way even now, they would not be A-bomb survivors, but people who do not understand RERF at all and are simply saying this."

Hayashida-san "In my opinion, those who insisted that ABCC treated A-bomb survivors as guinea pigs were people who led anti-ABCC campaigns. When I entered ABCC, no one said such a thing. At the beginning of the 1970s, pathological autopsies were performed at ABCC at both Nagasaki and Hiroshima facilities. This brought about a story to the effect that, when an A-bomb survivor died, ABCC cut the body into pieces and sent the data to the U.S. I don't believe such a story. However, the time when the pathological autopsy program was initiated was when people first started to say that ABCC treated A-bomb survivors as guinea pigs. I think hardly any one would say such a thing now."

Hayashida-san's comment prompted us to add *our own comment* on the subject, to the statements made by Moriyama-san, Inoue-san, and Mr. Rappaport during the earlier interview in Hiroshima, namely that the pathological autopsy program may have the root cause of such negative feelings about ABCC. Although clearly there was no intention of treating A-bomb survivors in any way, but with the utmost respect and dignity, the autopsy program seems to have produced this very regrettable effect.

What are your thoughts on RERF's current status and future activities? The organization is really at a "cross roads" and needs to develop long-term plans for its future. Over the past decade or so, there's been pressure to relocate RERF to a downtown area. What are your thoughts on this?

Inoue-san (on the Hijiyama site question) stated that "…relocation has been a long debated issue. Personally, it would be better to move RERF to the new downtown site…assuming of course that there is adequate financing of the move." "RERF studies should be continued for many more years, requiring new and improved facilities."

Moriyama-san indicated that the RERF Hiroshima lab should remain on Hijiyama. "ABCC made history on the Hijiyama site and these quonsets are historic, very unique, and famous buildings: everyone knows them in Hiroshima."

Mr. Rappaport commented that "…should the opportunity arise, RERF should move to a new, updated facility, because the present facilities are so old."

RERF is entering a critical period in terms of deciding what the organization will be 20, 30, 40 years from now. As the study cohorts are rapidly aging and diminishing in size, funding will become problematic. What's in RERF's future? A single purpose lab? A multiple purpose organization? Do you have any thoughts on this issue?

Inoue-san stated that he thought that the cohort studies should continue just as long as possible.

Moriyama-san added that these studies should not only continue, but be extended to include transgenerational studies on second and third generations.

Mr. Rappaport was a little more pragmatic in his response, by indicating the need to carefully evaluate the cost/benefit relationships of either extending the current work or pursuing new studies. However, he did say that the current studies needed

to be continued, but only within the current framework of the existing effort, and only until the long-term health effects of A-bomb irradiation in the survivors and their children are fully accounted for. As an aside, he commented that it must be difficult for the Department of Energy (DOE)/NAS to continue to support RERF when the competition for research funds is so great in the U.S. He also questioned the "…appropriateness and capacity of RERF to pursue alternative scientific fields of interest; studies of aging or lifestyle diseases do not seem to be a mission of RERF." He spoke about a woman who he knows that was diagnosed at ABCC with cancer of the cervix, and was counseled and guided on proper medical follow-up of her condition. Following hospitalization, and cancer surgery, she recovered. "This is what RERF should continue to be (as an organization)—helping survivors and their children through their radiation-associated medical problems."

Hayashida-san and **Okamoto-san** (on the issue of RERF's future direction) "As to the future, after the survivors are gone, I hope RERF continues as a research institute to study the possible genetic effects of parental A-bomb irradiation in the F_1 cohort."

A final charge was given to us "Do not forget to show the survivors who visited ABCC-RERF for exams that they are appreciated," suggesting that "…some of today's younger staff might not fully appreciate the contributions of the survivors."

Amen. We agree, we agree, we agree.

(Editors: Thomas Seed and Yuko Ikawa)

ABCC Quonset-hut buildings in Hiroshima in 1950

Nagasaki Prefectural Kyoiku Kaikan where ABCC was located in 1950

Present RERF Hiroshima facilities

Present building where Nagasaki RERF is located

Data Processing and Management System for Adult Health Study

Hiroaki Katayama, Chief, Department of Information Technology (ITD)

The Adult Health Study (AHS) is one of the major projects at RERF. AHS was created to collect disease incidence and health information through biennial medical examinations of the Life Span Study (LSS) survivors. The AHS examinations for a portion of this cohort make it possible not only to determine the full spectrum of human illnesses and physiologic disorders, and the incidence patterns for both cancers and non-cancer diseases in relation to radiation dose, but also to obtain important clinical and epidemiological information that are not accessible through mortality or cancer incidence follow-up of the full LSS cohort. In this article, we describe how the data from AHS cohort are actually collected and stored in the database.

The AHS was started in 1958, at a time when most of the office work was done by hand. For instance, staff of the Clinical Studies Department had to type the subject's name and address on each examination form. Also, all clinical data were coded manually by the Epidemiology Department, electronically transcribed onto floppy disks, and then eventually saved in the mainframe computer as a sequential text data.

The construction of the RERF network started in early 1987 using BRANCH 4670 (1 Mbps) and MS-NETWORKS. The network system was basically designed for the environment using a NEC mainframe computer. At the beginning of 1988, ITD started to build the AHS clinical database system. The ideas that drove the construction of this system were the following: first, to reduce the amount of manual clerical work required for the collection and recording of vital records; second, to have an electronic system that not only would minimize manual errors in the recording and retrieving of vital data, but also one in which essential data could be accessed more readily. The major problem in constructing such a system was the lack of a proper database system that was simultaneously shared by various sections of the Clinical Studies Department. There were database systems such as dBase IV and RBASE in the PC environment, however these were insufficient to meet the department's needs. Therefore, ITD had to create from scratch, an original database system and various application programs that could be used in various departmental sections. After the AHS clinical database was constructed, the department staff could now use the same data, for a multitude of purposes, that were managed in a single, unified database—a database that effectively serves to minimize clerical work associated with record keeping (e.g., eliminating the need to type the AHS participant's name and other information again and again for the different examination procedures and findings). Most clinical data, except some of the diagnosis and electrocardiograph data, were transferred automatically and directly to the database without the need of typing or coding by hand.

In 1997, we completely revamped the AHS database system. This was needed because of aging hardware (e.g., PCs and printers) coupled with increasingly heavy network traffic. We had to modify an old network system based on MS-DOS to Windows environment, as well as replacing the old AHS clinical database system with a new one using Sybase as a database engine.

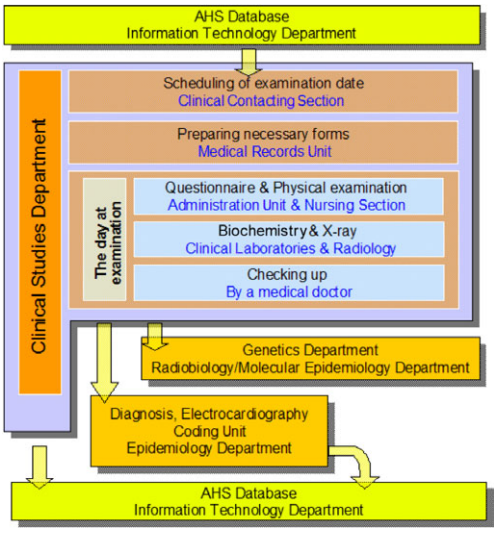

Figure. Adult Health Study data collection schema

The schematic of the AHS data collection is shown in the Figure. The first step of the AHS data collection is the scheduling of AHS subjects. The 20,000 AHS subjects are divided into A–Z groups, with specific groups being scheduled for clinical examinations every two years. The AHS clinical contact control list is produced from the AHS database according to this grouping. Based on this list, the Clinical Contacting Section makes a phone call

or a visit to a subject to fix the examination date, and puts the schedule into the AHS database. The contactor not only fixes the visit date and time, but also gets information such as a need for wheelchairs and other necessary items. This kind of information, which is not directly related to the research, is also put into the AHS clinical database. The Medical Records Unit in the Clinical Studies Department accesses the AHS database and prints all necessary forms for the clinical examination two or three days prior to the subject's scheduled date. The forms necessary for the examination are substantial, and amount to almost 50 pages for each AHS participant. The examination items are frequently added or deleted because of the start and finish of many substudies. Therefore, it is mandatory to produce the examination forms from blank paper in order to fully support the various components of ongoing AHS clinical and research activities. Furthermore, the Medical Records Unit prints two types of daily scheduling lists: one contains just subject's name, examination items and time, and the other contains additional, detailed items. The simple daily scheduling list is distributed to the Nursing Section and other related sections. The detailed one is used at the reception desk for the management of AHS subjects.

After the examinations are completed, the informed consent forms from the AHS subjects are preserved as medical record in medical charts. Other selected clinical data (e.g., oral temperature, blood pressure) are also entered into the database by the Clinical Administration Unit and the Nursing Section, and subsequently transmitted to the AHS database. Blood samples collected by the Nursing Section and processed by the Clinical Laboratories Division are separated into three tubes and stored at Departments of Clinical Studies, Genetics, and Radiobiology/Molecular Epidemiology, and those stored at the latter two departments are managed by the additional databases developed by ITD. Urine and stool specimen data collected using Hitachi HILAS system are also transferred to the AHS database, along with the results of any special examination such as ophthalmologic tests.

Two-dimensional labels on blood sample tubes serve to identify each sample, while securing and safeguarding donor anonymity.

X-ray images are transferred to the AHS database through FUJI FCR2000 by the Radiology Division. Osteoporosis measurement data are sent to the AHS database directly. All data regarding diagnosis, electrocardiographs, diagnoses of X rays and ultrasonography, pathological findings, and the osteoporosis questionnaire data are sent to the Coding Unit of Epidemiology Department. The data that have to be entered by hand are processed through a double data entry system with validity checks to avoid typographical errors. Numerous programs have been developed in order to accumulate and retain AHS data in a smooth and efficient manner. Following coding and data entry, these data are sent to the AHS database.

Upon completion of clinical examinations, an attending physician of the Clinical Studies Department documents the diagnoses and provides written comments. If the AHS participant needs more detailed examinations such as CT scanning or special therapy, the attending physician will write a letter of introduction for the participant for follow-up care at an appropriate medical institute or hospital. Finally, various reports produced from the AHS database will be sent by mail to the participant.

All tables in the AHS database are concatenated by the master file number. Although most clinical data will be transferred to the research database, the system id-number, which is given by the system automatically, is used to manage the resource database instead of the master file number. Therefore, researchers who analyze AHS clinical data cannot touch the master file number that directly connects to the personal identification. We are doing every effort to maintain confidentiality of such personal identification numbers. All application programs that are required to use the AHS database are supplied via network, only to the registered PCs equipped with the special ODBC driver. To access the AHS database, the specific username and password are required. They are different from those used to access the RERF network. The special encryption software is also installed into all PCs used to access the AHS database. The data contained in these PCs are encrypted, and staff cannot copy any file into the external media.

Since the AHS clinical examination is essential for RERF research, ITD always works closely and cooperatively with all the departments so that they can accomplish their research goals in a proper and timely fashion.

Research Protocols Approved in 2006

RP 1-06 Study on Cancer of the Uterus among A-bomb Survivors in the Life Span Study Cohort, 1950–2003 (Addendum to RP 8-85)

Tokuoka S, Fujihara M, Matsuo T, Nishisaka T, Nakajima H, Hirai Y, Nishi N, Soda M, Suyama A, Ikeda T, Ron E, Preston DL, Mabuchi K, Kodama K

Among the Life Span Study (LSS) cohort of the Radiation Effects Research Foundation (RERF), cases of cancer of the uterus (corpus and cervix) that developed during the period between 1957 and 2003 will be ascertained through the Hiroshima and Nagasaki tumor registries, supplemented by uterine cancer cases based on autopsy and surgical pathology records in major medical institutions in both cities and RERF file records and death certificate information from 1950 to 2003. Reported cases will be classified according to the International Classification of Diseases for Oncology (2003) codes and uterine cancers or misdiagnoses of uterine cancers will be reviewed by a panel of pathologists for histological diagnoses, and final diagnoses will be classified according to the World Health Organization's (WHO's) Histological Classification of Tumors (2003). Cases of the cervix classified histologically are investigated with polymerase chain reaction for human papillomavirus (HPV) infection and the viral type will be determined. The association between different types of HPV infection and cervical cancer will be investigated. To ascertain precancerous and early cancerous changes of the corpus (e.g., endometrial hyperplasia) as well as the cervix (cervical dysplasia), we will cooperate with medical institutions in Hiroshima and Nagasaki to identify and collect atypical hyperplastic endometrial lesions detected by clinical uterine scraping, or cervical dysplastic lesions identified by vaginal cytology, cervical biopsy or cervical conization among the LSS participants. We will investigate the association between estimated radiation doses (DS02) and cancers of the uterine corpus and cervix by confirmed histological types and sub-types.

RP 2-06 Relationship Between Radiation Exposure and Risk of Second Primary Cancers among A-bomb Survivors

Li CI, Nishi N, Sugiyama H, Soda M, Sakata R, Hayashi M, Kasagi F, Suyama, A, Mabuchi K, Davis S, Kopecky KJ, Kodama K

The primary goal of this study is to evaluate the relationship between radiation exposure and risk of second primary cancers among A-bomb survivors. The population base for this study is members of the Life Span Study (LSS), and the outcome of interest is the diagnosis of two primary cancers during follow-up. In addition to evaluating risk of second primary cancers by radiation dose, analyses will also be stratified by cancer type, treatments for the first cancer, gender, age at exposure, age at first cancer diagnosis, time since radiation exposure, and duration between first and second primary cancers. This study will provide greater insight into the effects of radiation exposure on cancer risk.

RP 3-06 Research Plan for RERF Studies of the Potential Genetic Effects of Atomic Radiation: Hiroshima and Nagasaki. Part 1. Mortality Study of Children of Atomic Bomb Survivors (Addendum to RP 4-75)

Suyama A, Kasagi F, Grant EJ, Cullings HM, Sakata R, Shimizu Y, Cologne JB, Kodama K

The "Ethical Guidelines for Epidemiological Research," revised as of 28 December 2004, stipulate the required items that should be described in a research protocol (RP). RP 4-75, "Research Plan for RERF Studies of the Potential Genetic Effects of Atomic Radiation: Hiroshima and Nagasaki," consists of five parts. The purpose of this addendum is to add required descriptions on items such as personal information protection and methods of obtaining informed consent to "Part 1. Mortality Study of Children of Atomic Bomb Survivors," one section of the RP. In addition, since much time has passed since RP 4-75 was approved, changes have happened to the conduct of the study. Thus, such changes and additions concerning "Part 1. Mortality Study of Children of Atomic Bomb Survivors" will be described.

Recent Publications

(Japanese): The original article is in Japanese.

Akashi S, Hara T, Takamura N, Imaizumi M, Ashizawa K, Akahoshi M, Aoyagi K, Yamashita S. Evaluation of thyroid function and iodine intake in Japanese young adults. Preparation for prophylaxis of stable iodide during radiation emergency. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):395-6. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Arataki K, Kumada H, Toyota K, Ohishi W, Takahashi S, Tazuma S, Chayama K. Evolution of hepatitis C virus quasispecies during ribavirin and interferon-alpha-2b combination therapy and interferon-alpha-2b monotherapy. Intervirology 2006; 49(6):352-61.

Arisawa K, Soda M, Akahoshi M, Fujiwara S, Uemura H, Hiyoshi M, Takeda H, Kashino W, Suyama A. Human T-cell lymphotropic virus type-1 infection and risk of cancer: 15.4 year longitudinal study among atomic bomb survivors in Nagasaki, Japan. Cancer Science 2006 (June); 97(6):535-9.

Ashizawa K. Epidemiology of Basedow disease and other thyroid diseases. Nippon Rinsho [Japanese Journal of Clinical Medicine] 2006 (December); 64(12 Special Issue):2194-200. (Japanese)

Asia Pacific Cohort Studies Collaboration (RERF: Nakachi K). An evaluation of metabolic risks for coronary death in the Asia Pacific region. Diabetes Research and Clinical Practice 2006 (December); 74(3):274-81.

Asia Pacific Cohort Studies Collaboration (RERF: Nakachi K). Central obesity and risk of cardiovascular disease in the Asia Pacific region. Asia Pacific Journal of Clinical Nutrition 2006; 15(3):287-92.

Asia Pacific Cohort Studies Collaboration (RERF: Nakachi K). The fraction of ischaemic heart disease and stroke attributable to smoking in the WHO Western Pacific and South-East Asian regions. Tobacco Controle 2006 (June); 15(3):181-8.

Cardis E, Howe G, Ron E, Bebeshko V, Bogdanova T, Bouville A, Carr Z, Chumak V, Davis S, ... Kopecky KJ, Kryuchkov V, Loos A, Pinchera A, Reiners C, Repacholi M. Shibata Y, Shore RE, et al. Cancer consequences of the Chernobyl accident: 20 years on. Journal of Radiological Protection 2006; 26:127-40.

Cologne JB. Re: When is baseline adjustment useful in analyses of change? An example with education and cognitive change (Letter). American Journal of Epidemiology 2006 (December); 164(11):1138-9.

Cullings HM, Fujita S, Funamoto S, Grant EJ, Kerr GD, Preston DL. Dose estimation for atomic bomb survivor studies: Its evolution and present status. Radiation Research 2006 (July); 166(1):219-54. (RERF Report 7-05)

Cullings HM, Levenson ZB, Funamoto S, Teranishi S, Noda A. Practical aspects of the DS02 dosimetry system for atomic bomb survivors. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2006 (June); 41(2):154-63. (Japanese)

Darby S, Hill D, Deo H, Auvinen A, Barros-Dios JM, Baysson H, Bochicchio F, Falk R, Farchi, Figueiras A, Hakama M, Heid I, Hunter N, Kreienbrock L, Kreuzer M, Lagarde F, et al. Residential radon and lung cancer—Detailed results of a collaborative analysis of individual data on 7148 persons with lung cancer and 14208 persons without lung cancer from 13 epidemiologic studies in Europe. Scandinavian Journal of Work, Environment & Health 2006; 32(Suppl 1):1-84.

Eguchi H, Nakachi K. Smoking as a risk factor for pancreatic cancer. Nippon Rinsho [Japanese Journal of Clinical Medicine] 2006 (January 28); 64:10-3. (Japanese)

Fujiwara S. Radiation effects on health among children of the A-bomb survivors. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):311-4. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Hagiwara N, Mechanic LE, Trivers GE, Cawley HL, Taga M, et al. Quantitative detection of p53 mutations in plasma DNA from tobacco smokers. Cancer Research 2006 (August 15); 66(16):8309-17.

Hakoda M, Kasagi F, Kusunoki Y, Matsuura S, Hayashi T, Kyoizumi S, Akahoshi M, Suzuki G, Kodama K, Fujiwara S. Levels of antibodies to microorganisms implicated in atherosclerosis and of C-reactive protein among atomic bomb survivors. Radiation Research 2006 (August); 166:360-6. (RERF Report 2-05)

Hamatani K, Eguchi H, Koyama K, Mukai M, Hayashi Y, Nakachi K. Application of improved SMART RACE method to RNA extracted from formalin-fixed and paraffin-embedded thyroid cancer tissue specimen: Detection of *RET*/*PTC8* in thyroid cancer among atomic-bomb survivors. Nagasaki Igakkai Zasshi [Nagasaki Medical Journal] 2006 (September 25); 81(Special Issue):346-9. (Proceedings of the 47th Late A-bomb Effects Research Meeting, 2006) (Japanese)

Hamatani K, Eguchi H, Takahashi K, Koyama K, Mukai M, Ito R, Taga M, Yasui W, Nakachi K. Improved RT-PCR amplification for molecular analyses with long-term preserved formalin-fixed, paraffin-embedded tissue specimens. The Journal of Histochemistry and Cytochemistry 2006 (July); 54(7):773-80. (RERF Report 13-05)

Hayashi T, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the *NKG2D* haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. Cancer Research 2006 (January 1); 66(1):563-70.

Hirai Y, Kodama Y, Moriwaki S, Noda A, Cullings HM, MacPhee DG, Kodama K, Mabuchi K, Kraemer KH, Land CE, Nakamura N. Heterozygous individuals bearing a founder mutation in the *XPA* DNA repair gene comprise nearly 1% of the Japanese population. Mutation Research 2006 (October); 601(1-2):171-8. (RERF Report 4-06)

Hirai Y, Kodama Y, Noda A, Nakamura N. Frequency of carriers bearing founder mutation at the XPA gene in a Japanese population. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2006 (June); 41(2):231-7. (Japanese)

Imaizumi M. Radiation exposure and thyroid disease in Hiroshima and Nagasaki atomic bomb survivors (Letter). Journal of the American Medical Association 2006 (August 2); 296(5):512-3.

Imanishi R, Seto S, Ichimaru S, Nakashima E, Yano K, Akahoshi M. Prognostic significance of incident complete left bundle branch block observed over a 40-year period. American Journal of Cardiology 2006 (September 1); 98(5):644-8. (RERF Report 11-05)

Inai K, Shimizu Y, Kawai K, Tokunaga M, Soda M, Mabuchi K, Land CE, Tokuoka S. A pathology study of malignant and benign ovarian tumors among atomic-bomb survivors—Case series report. Journal of Radiation Research 2006; 47(1):49-59. (RERF Report 4-05)

Iwamoto KS, Yano S, Barber CL, MacPhee DG, Tokuoka S. A dose-dependent decrease in the fraction of cases harboring M6P/IGF2R mutations in hepatocellular carcinomas from the atomic bomb survivors. Radiation Research 2006 (December); 166(6):870-6.

Kambe M, Matsumura M, Suyama A, Nishi N, Tsujiyama S, Hyoudou M, Yunokawa M, Murakami H, Urabe A, Yamane T, Arai H, Ono H, Watanabe T, Oda T, Soramoto E, Mukai M, Shimizu H, Nakamura A, Shimamura E, Yoshida Y, Usui S. Report on the results of the fifteenth medical examination of atomic bomb survivors resident in North America. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (January); 59(1):23-48.

Kanis JA, Johnell O, Oden A, Johansson H, Eisman JA, Fujiwara S, Kroger H, Honkanen R, Melton LJ, O'Neill T, Reeve J, Silman A, Tenenhouse A. The use of multiple sites for the diagnosis of osteoporosis. Osteoporosis International 2006 (April); 17(4):527-34.

Kanzaki H, Hanafusa H, Yamamoto H, Yasuda Y, Imai K, Yano M, Aoe M, Shimizu N, Nakachi K, Ouchida M, Shimizu K. Single nucleotide polymorphism at codon 133 of the *RASSF1* gene is preferentially associated with human lung adenocarcinoma risk. Cancer Letters 2006 (July); 238(1):128-34.

Kanzaki H, Ouchida M, Hanafusa H, Yano M, Suzuki H, Aoe M, Imai K, Shimizu N, Nakachi K, Shimizu K. Single nucleotide polymorphism of the *AXIN2* gene is preferentially associated with human lung cancer risk in a Japanese population. International Journal of Molecular Medicine 2006 (August); 18(2): 279-84.

Katayama H, Apsalikov KN, Gusev BI, Galich B, Madieva M, Koshpessova G, Abdikarimova A, Hoshi M. An attempt to develop a database for epidemiological research in Semipalatinsk. Journal of Radiation Research 2006; 47 (Suppl A):A189-97.

Kodama K. Long-term late effects of radiation on health. Houshasen Jiko Iryo Kenkyukai Kaiho [Bulletin of Japanese Association for Medical Management of Radiation Accident] 2007 (February 1); No.15:9-11. (Japanese)

Kondrashova TV, Neriishi K, Ban S, Ivanova TI, Krikunova LI, Shentereva NI, Smirnova IA, Zharikova IA, Konova MV, Taira S, Tsyb AF. Frequency of hemochromatosis gene (*HFE*) mutations

in Russian healthy women and patients with estrogen-dependent cancers. Biochimica et Biophysica Acta 2006 (January); 1762(1):59-65.

Kubo Y, Kusunoki Y, Hakoda M, Kasagi F, Yamaoka M, Sora M, Inoue M, Matsuura S, Hayashi T, Kyoizumi S, Fujiwara S, Akahoshi M, Nakachi K. Effects of atomic-bomb radiation on human immune responses. Report 22: Radiation-dose-dependent decreases in the percentages of CD4 T-cell subsets are also apparent for survivors in Nagasaki. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):409-12. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Kusunoki Y. Principles of flow cytometry (FCM). Diagnosis of hematopoietic tumors using flow cytometry. K. Muroi et al. (ed). Osaka: Iyaku Journal Co., Ltd. 2006 (August):pp 9-20. (Japanese)

Kusunoki Y, Hayashi T, Nakachi K. T-cell homeostasis and inflammatory response among A-bomb survivors. Low-Dose Radiation Exposures and Bio-Defense System (Proceedings on the International Symposium on Low-Dose Radiation Exposures and Bio-Defense System, 28-30 Sep. 2005, Aomori). Tanaka S, Fujikawa F, Oghiso Y (ed). Aomori: The Institute for Environmental Sciences 2006:pp 13-7.

Kusunoki Y, Yamaoka M, Kubo Y, Hamasaki K, Hayashi T, Kyoizumi S, Nakachi K. Effects of atomic-bomb radiation on human immune responses. Report 24: Measurements of memory CD4 T-cell subsets using CD43 expression lebels as markers. Nagasaki Igakkai Zasshi [Nagasaki Medical Journal] 2006 (September 25); 81(Special Issue):350-3. (Proceedings of the 47th Late A-bomb Effects Research Meeting, 2006) (Japanese)

Lagarde F. Understanding estimation of time and age effect-modification of radiation-induced cancer risks among atomic-bomb survivors. Health Physics 2006 (December); 91(6):608-18. (RERF Report 15-05)

Mizuno T, Tokuoka S, Kishikawa M, Nakashima E, Mabuchi K, Iwamoto KS. Molecular basis of basal cell carcinogenesis in the atomic-bomb survivor population: *p53* and *PTCH* gene alterations. Carcinogenesis 2006 (November); 27(11):2286-94.

Morishita Y, Hayashi T, Nagamura H, Kubo Y, Kasagi F, Kusunoki Y, Hakoda M, Kyoizumi S, Fujiwara S, Nakachi K. Effects of A-bomb radiation on human immunological response. 23rd report: Long-term elevation of inflammatory markers. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):413-6. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Nakachi K, Harris CC, Tahara E. Japan-US cooperative cancer research seminar on molecular epidemiological characteristics of lung and colon cancer development among atomic-bomb survivors, Bethesda, USA, February 23-24, 2006. Cancer Science 2006 (November); 97(11):1279-82.

Nakamura N. Genetic effects of radiation in atomic-bomb survivors and their children: Past, present and future. Journal of Radiation Research 2006 (October 1); 47 Suppl B:B67-73.

Nakamura N. Radiation effects on human health at high altitudes. Rinsho Kensa [Journal of Medical Technology] 2006 (November); 50(11):1229-31. (Japanese)

Nakamura N, Nakano M, Kodama Y, Ohtaki K, Niwa O, Toyoshima M. Chromosome aberrations do not persist in the lymphocytes or bone marrow cells of mice irradiated in utero or soon after birth. Hoshasen Kagaku [Radiological Sciences] 2006 (July); 49(7):225-9. (Japanese)

Nakanishi S, Yamada M, Tatsukawa Y, Fujiwara S, Yamane K, Kohno N. Relationship between BMI or HbA1c and mortality among atomic bomb survivors. Tonyobyo Gappeisho [Journal of Japan Society of Diabetic Complication] 2006; 20(2):126-9. (Japanese)

Nakashima E, Fujii Y, Neriishi K, Minamoto A. Analysis of postoperative cataract prevalence from atomic-bomb survivors with decision uncertainty. Nagasaki Igakkai Zasshi [Nagasaki Medical Journal] 2006 (September 25); 81(Special Issue):229-32. (Proceedings of the 47th Late A-bomb Effects Research Meeting, 2006) (Japanese)

Nakashima M, Miura S, Kondo H, Hayashi T, Wen CY, Kawai K, Soda M, Matsuo T, Sekine I. Discrimination of multiple primary cancers from cancers in multiple sites among atomic bomb survivors. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):341-3. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Nakayama T, Kuroi N, Sano M, Tabara Y, Katsuya T, Ogihara T, Makita Y, Hata A, Yamada M,

Takahashi N, Hirawa N, Umemura S, Miki T, Soma M. Mutation of the follicle-stimulating hormone receptor gene 5′-untranslated region associated with female hypertension. Hypertension 2006 (September); 48(3):512-8.

Neriishi K. Rehabilitation program for residents at radiation contaminated area caused by Chernobyl accident. Houshasen Jiko Iryo Kenkyukai Kaiho [Bulletin of Japanese Association for Medical Management of Radiation Accident] 2007 (February 1); No.15:15-6. (Japanese)

Neriishi K, Nakachi K, Sharp GB, Eguchi H, Grant EJ, Cologne JB, Land CE, Stevens RG, Izumi S. Radiation effects on sex hormones and growth factors in postmenopausal A-bomb survivors: Fundamental analysis in controls of a case control study of breast cancer in atomic bomb survivors. Nagasaki Igakkai Zasshi [Nagasaki Medical Journal] 2006 (September 25); 81(Special Issue):233-9. (Proceedings of the 47th Late A-bomb Effects Research Meeting, 2006) (Japanese)

Neriishi K, Nakashima E, Fujiwara S, Akahoshi M. Postoperative cataract in atomic-bomb survivors; Dose response relationship, indirect effect factors and threshold. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):330-2. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Nishi N, Sugiyama H, Kodama K, Okuno H, Kuwabara M, Hiramatsu K, Arita K, Yasui W, Usui S. Mesothelioma as seen in tumor registry in Hiroshima prefecture and Hiroshima City. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (May); 59(5):435-8. (Japanese)

Ohishi W, Chayama K. Diagnosis of and countermeasures for variant strain development. Modern Medical Treatment of Liver Disease. Toda G, et al. (edit). Tokyo: Advanced Medical Technology Research Institute 2006 (April):pp 164-9. (Japanese)

Ohishi W, Fujiwara S, Suzuki G, Kishi T, Sora M, Matsuura S, Hakoda M, Yamada M, Chayama K. Feasibility of freeze-dried sera for serological and molecular biological detection of hepatitis B and C viruses. Journal of Clinical Microbiology 2006 (December); 44(12): 4593-5. (RERF Report 8-05)

Rogounovitch TI, Saenko VA, Ashizawa K, Sedliarou IA, Namba H, Abrosimov AY, Lushnikov EF, Roumiantsev PO,Konova MV, Petoukhova NS, Tchebotareva IV, Ivanov VK, Chekin SY, Bogdanova TI, Tronko MD, Tsyb AF, Thomas GA, Yamashita S. *TP53* codon 72 polymorphism in radiation-associated human papillary thyroid cancer. Oncology Report 2006 (April); 15(4):949-56.

Seto S, Nagao S, Ozeki S, Tetsuo H, Akahoshi M, Yano K. Contribution of central nitric oxide to the regulation of blood pressure and sodium balance in DOCA-salt hypertension. Journal of Cardiovascular Pharmacology 2006 (May); 47(5):535-9.

Seto S, Soda M, Nakashima E, Yano K, Akahoshi M. Longitudinal analysis of blood pressure trends and prognosis in isolated systolic hypertension in elderly individuals. American Journal of Hypertension 2007 (February); 20(2):134-9. (RERF Report 12-05)

Sharp GB, Mizuno T, Fukuhara T, Tokuoka S. Lack of association between acute exposure to ionizing radiation and liver cirrhosis. International Journal of Radiation Medicine 2006 (April); 82(4):231-40. (RERF Report 11-03)

Shimizu Y. Solid cancer risk. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):298-301. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Suyama A, Kasagi F, Grant EJ, Nishi N, Kodama K, Cologne JB, Furukawa K, Akahoshi M, Fujiwara S, Shore RE. Health effects study of the children of A-bomb survivors (Abstract). American Journal of Epidemiology 2006 (June 1); 163(11):S253. (2006 Congress of Epidemiology Abstracts)

Suyama A, Kodama K, Kasagi F, Shimizu Y, Soda M, Nishi N, Sugiyama H, Grant EJ. Future issues for the Radiation Effects Research Foundation in the study of risks from A-bomb radiation. Nagasaki Igakkai Zasshi [Nagasaki Medical Journal] 2006 (September 25); 81(Special Issue):157-64. (Proceedings of the 47th Late A-bomb Effects Research Meeting, 2006) (Japanese)

Takahashi K, Eguchi H, Arihiro K, Ito R, Koyama K, Soda M, Cologne JB, Hayashi Y, Nakata Y, Nakachi K, Hamatani K. The presence of *BRAF* point mutation in adult papillary thyroid carcinomas from atomic bomb survivors correlates with radiation dose. Molecular Carcinogenesis 2007 (March); 46(3):242-8. (RERF Report 3-06)

Takahashi N, Sasaki K, Kodaira M, Satoh Y, Kodama Y, Sugita K, Katayama H, Tsuyama N. Study for genetic effects of atomic-bomb radiation by using

of a DNA microarray-based comparative genomic hybridization (array-CGH) method—Summary of preliminary study. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2006 (April); 59(4):417-21. (Proceedings of the 46th Late A-bomb Effects Research Meeting, 2005) (Japanese)

Ueda H, Ito M, Eguchi H, Tanaka S, Yoshihara M, Haruma K, Hatakeyama M, Chayama K. Development of a novel method to detect *helicobacter pylori cagA* genotype from paraffin-embedded materials: comparison between patients with duodenal ulcer and gastric cancer in young Japanese. Digestion 2006; 73(1): 47-53.

Yamada M. Effects of the atomic bomb exposure on health condition among survivors. RERF Adults Health Study. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2006; 41(2):164-74. (Japanese)

Publications Using RERF Data

The following publications represent research done by non-RERF scientists based on the data publicly available from RERF.

Sasaki MS, Endo S, Ejima Y, Saito I, Okamura K, Oka Y, Hoshi M. Effective dose of A-bomb radiation in Hiroshima and Nagasaki as assessed by chromosomal effectiveness of spectrum energy photons and neutrons. Radiation and Environmental Biophysics 2006 (July); 45(2):79-91.

Tomita M, Otake M, Moon S. Statistical assessment on cancer risks of ionizing radiation and smoking based on Poisson models. Journal of the Korean Data & Information Science Society 2006 (May); 17(2):581-98.